4 Cellular Brain Ischemia and Stroke: Neuroprotection, Metabolism, and New Strategies for Brain Recovery

Kelley A. Foster, Christopher J. Beaver, Larry B. Goldstein, and Dennis A. Turner

CONTENTS

4.1 INTRODUCTION

Each year 4.6 million people die from stroke worldwide and 75% of these cases occur in industrialized countries.[1] In the U.S., stroke is the third leading cause of mortality, with 4.7 million survivors, 15 to 30% of whom are left with permanent disabilities and 20% of whom require long-term institutional care.[2] Significant social, financial, and personal problems occur as a result of these disabilities.[3] *Stroke* is a generic term, encompassing a wide variety of vascular diseases affecting the nervous system. Treatment of these diverse disease processes necessarily involves several different approaches.

Because the brain relies completely on a constant supply of oxygen and glucose for normal function, ischemic injury can occur rapidly if the delivery of these substrates is impaired as a result of transient or permanent cessation of blood flow. Such ischemic injury occurs in nearly 80% of stroke cases due to occlusion of either a major proximal or cerebral artery, most commonly as a result of an embolus or local thrombus. The remaining causes of stroke relate primarily to bleeding in or around the brain.

Acute revascularization and neuroprotective strategies have been the two most extensively studied specific approaches to the treatment of acute ischemic stroke. Of the 178 controlled clinical trials of acute stroke therapies conducted in the past century, only trials of intravenous tissue plasminogen activator (tPA) have been sufficiently positive to lead to approval by the U.S. Food and Drug Administration.[3,4]

Despite showing promise in preclinical studies, none of the more than 114 stroke trials that examined more than 49 neuroprotective drugs have been positive.[4] This discrepancy between preclinical data and the results of clinical trials illustrates the significant challenge of translational neuroscience. These difficulties may have arisen from the use of unsuitable preclinical animal models, inappropriate extrapolation of preclinical data to human trials, or poor clinical trial design.[5,6] However, these multiple failures and experiences can provide useful information to help guide new translational approaches to stroke therapy.

4.2 TYPES OF STROKES AND CEREBRAL ISCHEMIA EVENTS

Causes of ischemic stroke include extracranial or intracranial steno-occlusive disease affecting large- or medium-sized arteries most frequently related to atherosclerosis, embolization from a cardiac or arterial source, and occlusion of small intracranial vessels.[7] In up to 40% of cases, the cause is unknown or the stroke is due to multiple

possible etiologies. Atherosclerosis occurs as a result of a complex series of processes leading to arterial injury with cholesterol deposition. Atherosclerotic plaques can provide a nidus for platelet aggregation and thrombus formation, or they can rupture. They can then occlude the artery at the site of clot formation or lead to emboli that can block a distal vessel.

A variety of cardiac conditions can lead to embolization. They include arterial fibrillation, valvular heart disease, ventricular or septal aneurysm, and cardiomyopathies. Small vessel intracranial disease is most frequently associated with hypertension and leads to ischemia in the distribution of penetrating arteries, resulting in so-called "lacunar" syndromes. A large number of other less common conditions including arterial dissection, nonatherosclerotic vasculopathies, hypercoagulable states, and hematological disorders can also lead to ischemic stroke.

Temporary focal ischemia (transient ischemic attacks or TIAs) may also occur. TIAs are traditionally defined as producing neurological symptoms lasting less than 24 hours, but most are far shorter. They are not only harbingers of ischemic stroke, but may also reflect cerebral infarction with transient symptoms (i.e., stroke with rapid functional recovery).[8] Other less common causes of stroke include intracerebral hemorrhage and subarachnoid hemorrhage (SAH).

SAH usually results from rupture of saccular aneurysms most commonly located at branch points in major arteries at the base of the brain. SAH can cause the subarachnoid space to fill with blood at nearly arterial pressure, resulting in direct brain injury due to decreased perfusion of the brain. The presence of blood around major vessels also can lead to delayed cerebral vasospasm (see Chapter 11), then to delayed ischemic stroke due to vessel narrowing and lack of perfusion.[9]

In contrast to focal ischemia caused by arterial occlusion, global ischemia can result from other types of conditions, such as cardiac arrest, near-drowning, or hypotension. Depending on its severity and on other factors, less than 5 minutes of global ischemia can be tolerated before lasting damage occurs.[10] Cerebellar Purkinje cells, CA1 hippocampal pyramidal neurons, and layers 3 and 5 of the neocortex are relatively more vulnerable to global ischemia than other areas of the brain.[10]

4.3 CELLULAR CONSEQUENCES OF STROKE

Although the brain only comprises 2.5% of body weight, it accounts for nearly 25% of basal metabolism. Neuronal function and survival are highly dependent on aerobic metabolism.[11] When a cerebral artery becomes occluded, the lack of oxygen and glucose rapidly leads to neuronal death unless blood supply is restored. However, before this final stage takes place, a cascade of multiple biochemical events is initiated and includes the interactions of a number of different cells in the ischemic area, including neurons, mitochondria, astrocytes, fibroblasts, smooth muscle cells, endothelial cells, and blood components.[12–14] The process begins with the impairment of energetics required to maintain ionic gradients (see Figure 4.1).[15] With the loss of membrane potential, neurons and glia become depolarized,[16] which in turn activates voltage-dependent Ca^{2+} channels. This activation leads to the release of excitatory amino acids into the extracellular space.

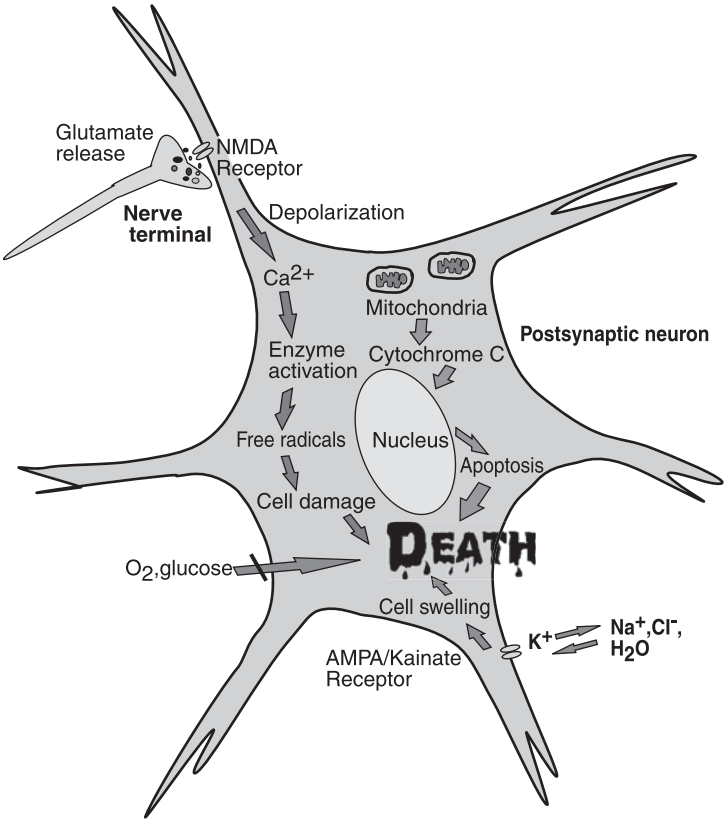

FIGURE 4.1 (See color insert following page 146.) Mechanisms of cell death. A typical neuron is represented indicating a variety of perturbed physiological mechanisms leading to cell death. These mechanisms include excess glutamate stimulation and secondary depolarization (excitotoxicity); loss of substrate (oxygen or glucose); free radical formation, particularly following reoxygenation; apoptosis initiated by cytochrome C release from mitochondria; and cell swelling induced by water influx.

Excitatory amino acids further accumulate because their presynaptic uptake is energy dependent. This can lead to further injury in ischemic neurons that otherwise might remain above the threshold of viability. Activation of excitatory amino acid receptors leads to further sodium and calcium entry.[17] Several different types of excitatory amino acid receptors have been identified pharmacologically. The N-methyl-D-aspartate (NMDA) receptors are gated channels that are highly permeable to Ca^{2+}. Ca^{2+} accumulation is also triggered secondarily by Na^+ influx through α-amino-ε-hydroxy-5-methyl-4-isoxazole-propionic acid (AMPA)-, kainate-, and NMDA-receptor gated channels through activation of voltage-gated Ca^{2+} channels and reverse operation of the Na^+/Ca^{2+} exchanger.[17,18]

Na^+ and Cl^- enter the neurons via monovalent ion channels (such as AMPA- or kainate-receptor gated channels) as a result of glutamate-mediated overactivation.

Inhibitory neurotransmitters (primarily gamma aminobutyric acid [GABA]) are important to slow the excitatory cascade; these neurotransmitters result in chloride flux into cells. The overall effect of these changes in Na and Cl ionic gradients is the passive influx of water leading to cellular edema.

At the same time, K^+ exits the neurons as part of the inhibitory currents following action potentials. In conjunction with the accumulation of extracellular glutamate, increased K^+ levels can promote repeated neuronal depolarizations in the penumbral regions (see later discussion). These "peri-infarct depolarizations" are closely related to hypoxic spreading depression or anoxic depolarization.[19] Frequent neuronal depolarizations result in increased metabolic demands because of severe neuronal membrane depolarization, further worsening ischemic injury and increasing the zone of frank infarction.[19,20]

The accumulation of calcium also initiates a cascade of processes leading to delayed tissue damage. For example, calcium induces proteolytic enzymes, which degrade cytoskeletal proteins. It activates enzymes that lead to the formation of free radical species causing lipid peroxidation and membrane damage. Oxygen free radicals can also promote inflammation and apoptosis. Mitochondria are significant sources of oxygen free radicals that can damage mitochondrial membranes. Oxidation impairs the function of mitochondrial proteins that participate in adenosine triphosphate (ATP) production, electron transport, and H^+ extrusion.[21] Accumulation of calcium and free radical formation in mitochondria favors the formation of permeability transition pores that induce cell death.[22]

Neurons permanently lose membrane potential when blood flow drops more than 20% below normal for more than a very short period.[23] This ischemic core region is surrounded by an area known as the penumbra that is characterized by more modest reductions in blood flow and associated with impaired neuronal function.[24] Although the core of infarcted tissue is not salvageable, the ischemic penumbra partially preserves energy metabolism[25] that may be either reversible or may proceed on to infarction as a result of the cascade of processes including those previously discussed.[26]

Programmed cell death (apoptosis) and excitotoxic necrosis can occur simultaneously in the ischemic brain.[27,28] Apoptosis is a genetically regulated program in which protein-cleaving enzymes known as caspases promote cell death. Caspases 1 and 3 seem to be the predominant proteins involved.[26] Mitochondria release cytochrome C, which induces apoptosis.[29] Several factors determine whether apoptotic or delayed excitotoxic cell death predominates. The factors include the maturity of the neurons, the extent of the injury, the availability of trophic support, and the intracellular free calcium concentration.[30]

In addition to these mechanisms, the accumulation of free radicals and calcium-activated intracellular second messenger systems produces inflammatory mediators such as platelet-activating factor, tumor necrosis factor-α, and interleukin-1β.[13] These inflammatory mediators activate microglia and result in leukocyte infiltration through an increase in endothelial adhesion molecules.[26] As a result of the interactions of complementary receptors on neutrophils and adhesion molecules, the neutrophils adhere to the endothelium, travel through the vascular wall, and enter the brain parenchyma. Post-ischemic inflammatory processes can

also contribute to secondary neuronal injury and final infarct size[31,32] through a number of mechanisms including microvascular obstruction by neutrophils[33] and the production of toxic mediators.

Zinc may also be an important mediator of secondary neuronal injury. Under normal physiological conditions, zinc modulates the action of NMDA-receptor gated calcium channels and is critical for the action of several metalloenzymes and transcription factors. Zinc is variably released from excitatory nerve terminal vesicles upon normal synaptic functioning. During ischemia, zinc is thought to be excessively released across the plasma membrane through a number of mechanisms including: activation of voltage-gated calcium channels, NMDA-receptor gated channels, transport exchange for intracellular Na^+, and Ca^{2+}-permeable AMPA receptors.[34] Zinc accumulates in neuronal cell bodies after its release from synaptic terminals.[35] The release of zinc causes apoptosis or necrosis, depending on the extent of the exposure, possibly through direct inhibition of aerobic glycolysis and depletion of energy.[30]

Preclinical animal studies show that the time between initiation of ischemia and the delivery of a putative neuroprotective drug is critical.[36–39] Depending on experimental conditions, neurons occupying the bordering areas of the ischemic territory may be able to survive up to 48 hours following ischemic insult.[40] However, the therapeutic window is considerably shorter.

Understanding of the pathophysiological mechanisms involved in ischemic injury led numerous research groups to develop possible treatments targeting various steps of the cascade. Over the past 10 to 15 years, several animal models of both focal and global ischemia have been developed in attempts to simulate the neuropathological consequences of human stroke. The majority of the early treatments sought to modulate the initial metabolic events following ischemia, in particular excitotoxic mechanisms by using a variety of NMDA receptor antagonists and calcium channel blockers.[12,41] Free-radical scavengers, caspase inhibitors and GABA agonists have also been evaluated. Although results in animal models are promising, all attempts to translate these findings into an efficacious clinical treatment have failed.[42,43]

4.4 PROBLEMS IN TRANSLATION OF STROKE TREATMENTS FROM BENCH TO BEDSIDE

Aside from the neuroanatomical, pathophysiological, pharmacokinetic, and genetic differences among laboratory animals (in particular rodents) and humans, fundamental differences also exist in the designs of preclinical studies and clinical trials:[44] (1) treatment window following stroke, (2) target area of the brain (gray versus white matter), (3) duration of drug treatment, (4) pharmacokinetics, and (5) outcome measures. Laboratory studies are tightly controlled; whereas, human clinical trials involve heterogeneous subjects. Because no approach has yet been successful, the type of preclinical studies that are sufficient to warrant proceeding to clinical trials remains uncertain.

4.4.1 Animal Models of Ischemic Stroke

A variety of animal models have been used to study ischemic stroke to evaluate potential therapies.[45] The most frequently used models of global ischemia involve either bilateral carotid artery occlusion in gerbils or bilateral carotid occlusion with hypotension or four-vessel occlusion in rats. Models of focal ischemia have been developed in a number of animal species and can involve transient or permanent arterial occlusion.

The damage following permanent occlusion results in an ischemic core area surrounded by a penumbral region of varying size. The middle cerebral artery (MCA) occlusion model is among the most commonly employed.[46] An intraluminal thread is used to cause the vessel occlusion and can be withdrawn after 1 to 2 hours to mimic reperfusion or can be left in place to cause permanent occlusion. Experimental factors such as trauma, temperature regulation, stress, and anesthetic use (some of which can have neuroprotective effects alone or in combination with experimental drugs[47]) may complicate interpretation of the results.

Animal models have greatly aided our understanding of the ischemic penumbra and other pathophysiological mechanisms of stroke.[48] Although the results from animal experiments have provided the principles guiding the design of human clinical trials, the results should be used with caution.

Many laboratory animal models are intended to explain basic pathophysiological mechanisms of ischemia and have not been validated for predicting drug efficacy in humans. This is because of a number of important differences between animal models and human strokes. For example, the infarct volume resulting from occlusion in animal models is both uniform and reproducible, and therefore does not necessitate the need for large sample numbers. Experimental conditions such as body temperature, glucose levels, blood pressure, acid-base balance, and oxygenation are tightly regulated and may alter an animal's response to an ischemic insult. In contrast, human stroke is a highly variable clinical condition as a result of differences in location, cause, severity, and reversibility. Stroke types vary considerably in humans (cortical, mixed cortical–subcortical, pure subcortical, white matter, or ischemic and hemorrhagic strokes).

Most animal stroke models use lissencephalic species such as rodents (humans are gyrencephalic). Animal models do not generally consider co-morbid disease states such as diabetes, hypertension, and infections.[44] Humans typically receive a number of different drugs to treat co-morbid conditions that alter the underlying milieu as compared to experimental conditions. In addition, animals used in stroke models are most commonly young as compared to the typically aged human who is afflicted with stroke. All these variables limit extrapolation from the animal results, even when a study considers the same stroke type in preclinical and clinical situations.

Changes in some of the methodology used in laboratory models can make them more relevant to human stroke. First, the occlusion should be transient so as to enable entry of the drug to the site of injury. This would also better reflect the condition in humans in which varying degrees of perfusion are reestablished through collaterals or clot lysis. Drugs should be evaluated in a number of animal species

and models to support the generalizability of their purported effects. Allocation of treatment and outcome assessment should be blinded or masked to avoid potential bias. Experiments should be carried out in aged animals to match the human ages commonly observed in stroke patients. Assessment of functional behavioral outcomes in addition to structural outcomes, such as volume of infarction, is essential because functional outcome is the basis of clinical trial assessment. Outcome assessment should be delayed as long as feasible based on animal species. Human trials generally conduct outcome assessments at least 3 months after stroke.

4.4.2 Time Windows of Treatment

With the advent of thrombolytic therapy, stroke is now considered an emergency condition with the same priority as acute myocardial infarction. Many hospitals have developed acute stroke teams, and communities are being organized to facilitate the rapid transportation of stroke patients to appropriate facilities. In addition, efforts have been made to increase public and professional awareness of stroke. People at risk of stroke and their families and friends should be alerted to the common symptoms of stroke.[49]

Unlike rigorously controlled preclinical studies, the time taken to arrive at a hospital following stroke and therefore the time at which the patient is available for treatment after the actual onset of ischemia varies. Between 1995 and 1999, the median time to entry into an acute stroke clinical trial was 14.3 hours, compared to a median permitted entry window of 12 hours.[4] Past studies suggest that irreversible focal injury takes place after only a few minutes and is complete after 6 hours.[10] Although individuals may have salvageable tissue up to 6 hours or longer following a stroke, the progression of damage varies among patients and depends on collateral circulation and other factors.[50]

The failures of past neuroprotectant trials may have in part been due to the administration of the putative neuroprotectants after irreversible injury had occurred. Therefore, potential neuroprotectants must be tested at realistic time points in preclinical studies, but at time intervals longer than minutes. A drug that is efficacious in animal models only if given immediately after arterial occlusion is unlikely to be of benefit. For example NXY-059, a novel nitrone, is effective when administered 3 to 6 hours following recirculation in transient focal MCA occlusion models[51] and at 4 hours in permanent focal MCA occlusion models.[52] Therefore, it would not be reasonable to initially test this drug in humans beyond 6 hours.

4.4.3 Early vs. Late Outcome Definition

Preclinical studies have commonly used histological endpoints to assess therapeutic efficacy. These histological outcomes (i.e., reduction in infarct size) have been generally assessed between 48 and 96 hours. However, ischemic injury can continue to develop for weeks or even months.[53] As a result, early histological endpoints can lead to erroneous conclusions. For example, MK-801 appeared to reduce infarct size at 3 days following ischemic insult but the benefit was not significant after 4 weeks.[54] A number of other drugs including SNX-111 (N-type calcium channel antagonist),

NBQX (AMPA antagonist), and flavopiridol (cyclin-dependent kinase inhibitor) showed potential neuroprotection 1 week following ischemia, but had no effect if the assessment was carried out 4 weeks post-insult.[55,56]

In comparison to preclinical animal studies in which injury is assessed histologically at early time points, clinical trials rely on behavioral and functional outcomes at later stages (generally at 3 months following stroke)[4,57] to assess the effectiveness of intervention. Early behavioral assessments are suggested to be more predictive than histological endpoints.[58] For example, some drugs may be effective in improving functional outcome but may not reduce the resulting infarct size, suggesting that the drugs are acting via other mechanisms.

Such mechanisms may include stimulation of neuronal sprouting and protection against retrograde neuronal death.[59,60] Therefore, in addition to infarct size assessments, preclinical studies should include functional measures of motor, sensory or cognitive deficits in order to gauge the therapeutic efficacy.[61,62] A large variety of tests have been developed for this purpose (see Gladstone et al.[44] for references). Recent preclinical studies have employed complex behavioral tasks as endpoints for determining whether the treatment in question will aid in the reduction of ischemia-related disability.[63,64]

4.4.4 Regional Differences in Target Areas of Brain

Preclinical neuroprotectant studies have targeted the ischemic penumbra. However, in some patients the penumbra may only account for a small percentage of the total infarct volume. To increase the likelihood of detecting a drug effect, clinical studies should target patients with sufficiently large penumbrae.[6,50] However, the optimal way of detecting the penumbra in the context of a clinical trial has not been fully established, and no treatment has been proven efficacious with the use of this approach.

Past clinical trials tended to treat stroke as a single disease entity. Only 62 (35%) of the 178 published stroke trials specified a particular stroke territory (e.g., carotid artery, MCA).[4] The majority of drug therapies tested in animal models targeted gray matter. In comparison to the rodent brain, the human brain contains a higher proportion of white matter (including axons) that may not be salvageable using therapeutic agents that only target gray matter.[65–67] Approximately a third of strokes involve deep white matter and may not respond to neuroprotective therapy. It is therefore possible that potentially successful neuroprotectants have failed due to the inclusion of patients with white matter injuries in clinical trials. Clinical trials should limit selection to patients most likely to benefit. This is particularly important for Phase II clinical trials that provide data critical for a decision to proceed with or defer a large Phase III efficacy study.[5]

4.4.5 Pharmacokinetics, Safety Issues, and Appropriate Dosing

In order for a drug to be effective as a neuroprotectant, it must satisfy a number of criteria. First, it must be able to reach the target region and cells within the brain. Because the blood–brain barrier (BBB) is often damaged by the ischemia to variable

extents at different times, some drugs normally excluded from the brain may still be able to reach target tissue, but this is likely to be variable.

Drugs that can cross the BBB may in some conditions have preferred access, depending on diffusion and vascular stasis; to cross the BBB, a drug must be lipid-soluble and have a molecular weight below 500 Da.[68] Recombinant proteins, monoclonal antibodies, gene therapy, and antisense drugs, all of which could serve as potential neuroprotectants, are too large to cross from the systemic circulation into the brain and may require direct infusion into the brain for effective delivery. Because no gene or drug targeting strategies are clinically available, drug testing is now limited to small lipid-soluble drugs that represent only 2% of all potential candidates for drug development.[68]

However, even this limited class of drugs may still exhibit poor access to the critical brain regions targeted. Because of the vascular occlusion, access to the ischemic region is likely to be reduced. Thus, directly sampling the area of brain targeted for drug levels may be a critical control to evaluate whether access into the critical region is possible. After this critical initial point is established, mechanisms of action may be thoroughly assessed by histological and behavioral outcomes.

Dosing is another important consideration. The neuroprotectant dose of a drug in animal models may result in intolerable toxicity in humans. Testing these drugs at doses below those required for efficacy in animal models is less likely to be successful in humans. Duration of treatment must also be considered. Although a drug exhibits neuroprotective properties after a single dose, multiple doses over the period in which the infarct is evolving may or may not increase its clinical efficacy.[69,70] The lengths of treatment have varied from a single injection, to continuous infusions, to several doses extending 3 months after a stroke.[69] Full dose response studies must be performed to avoid problems associated with an inverted U-shaped dose response curve.[71]

Several factors may influence the doses required for therapeutic treatment. If given by constant intravenous infusion, a lipid-soluble drug will accumulate in the cerebral tissues faster than a hydrophilic drug and will take longer to clear from the tissues. This delay may lead to increased toxicity. Therefore, plasma-level calculations may overestimate the levels needed for *in vivo* activity. Other considerations include the receptor-binding properties of the drug that will determine the loading dose and the need for maintenance infusion, the clearance and volume of distribution of the drug, and its therapeutic index.[69]

The duration of therapy is also influenced by side effects the drug might produce and pathophysiological changes following the stroke. For example, the more potent NMDA antagonists produce psychomimetic effects that might preclude the drug from administration over days to weeks.[69] As a result of the loss of autoregulation in acute stroke, drugs that produce hemodynamic effects may increase or decrease cerebral blood flow which in turn could exacerbate edema or worsen ischemia. Moderate increases in blood pressure, however, could be beneficial in improving blood flow and local perfusion.[69] In addition, these drugs may be used to increase the initial time window during which longer lasting drugs may be administered.[56]

4.4.6 Clinical Outcome Measures and Statistical Issues with Clinical Trials

A variety of measures have been used to assess outcomes in clinical stroke trials. Less than half of the published clinical trials utilized validated outcome measures and only 17% indicated primary endpoint.[4] The choice of outcome measures can play a fundamental role in whether a therapeutic agent is deemed successful.[6,57,72] Outcome can be assessed at the level of impairment (NIH Stroke Scale), disability (Barthel Index), or social handicap (Rankin Index). In addition, although they are not yet widely employed, scales that incorporate quality-of-life assessment and pharmacoeconomic analysis can be used as secondary outcome measures.[73] Final outcome assessments are generally carried out at least 3 months after stroke as recovery has usually reached a plateau by that time.

The use of a dichotomous division of a continuous scale may help determine whether a patient has achieved a clinically significant benefit.[73] Global statistics can take into account multiple assessment scales and can be used to provide overall assessments of benefits.[74] The National Institute of Neurological Disorders and Stroke's rtPA trial[75] utilized this approach.

Statistical power is an important consideration for clinical trial design. Studies must have sufficient statistical power to ensure that a lack of a treatment effect results from a lack of biological effect of the intervention and is not due to insufficient sample size. Kidwell et al.[4] calculated the sample sizes required for a 5% reduction in the proportion of patients dead or disabled at 6 months as 3148 (reduction from 60% to 55%; 80% power, alpha = 0.05). The mean sample size per trial of the 178 controlled clinical trials for acute ischemic stroke performed up until 1999 was 415 patients. The mean sample size for neuroprotective trials was 186 patients (median 69). Potentially efficacious drugs might have been abandoned because of a type II statistical error.[4] The Stroke Therapy Academic Industry Roundtable has developed a series of recommendations for translating preclinical studies into clinical trials based on these reviews and other considerations.[5,6,76]

4.4.7 Clinical Trials

Following successful outcomes in animal models, a drug may be assessed further in human clinical trials that consist of three phases. Phase I trials are conducted in healthy volunteers to determine whether untoward toxicity is present and to evaluate the maximal tolerated dose. Phase II studies are performed in persons who have the disease and include questions focused on dose finding, safety, and potential efficacy. Phase III trials are large-scale studies with sufficient statistical power to assess efficacy.[73]

Phase I trials are often conducted in young healthy volunteers. In contrast, stroke patients are most frequently elderly, where age-related changes in cerebral dynamics and vasculature can significantly affect toxicity as well as pharmacokinetics and regional cerebral blood flow. Therefore, the inclusion of healthy elderly patients in Phase I trials may help avoid under-recognition of potential side effects in the eventual target population.[73]

Phase II and III trials are sometimes combined to reduce the numbers of patients who need to be included and save time.[77] This results in having to use preclinical and Phase I data to develop a protocol for clinical efficacy.[73] Phase II trials are sometimes divided into IIa and IIb studies.[6] Phase IIa studies often focus on providing initial toxicity data and exploring dosing and pharmacokinetic issues. Phase IIb trials are important for refining patient selection, dose, route, timing, duration of therapy, and for better understanding of side effects, pharmacokinetics, and drug interactions.[6]

4.5 FUTURE POTENTIAL TREATMENTS AND OPPORTUNITY TIME WINDOWS

The consequences of acute ischemic injury evolve over time, and drug treatments corresponding to various successive events within the ischemic cascade may be developed in the future. A "cocktail" of therapies may need to be developed and tested to address these potential overlapping therapeutic windows.[6] Combination therapy may also reduce the dose-limiting toxicity encountered in the use of single agents if multiple agents can be administered at lower doses. In some cases, the administration of a second drug may improve the action of the first. For example, the combination of a thrombolytic agent and a neuroprotectant may increase the chances of the latter drug reaching the site of injury within the required time window.[73]

Combination therapy may also offer synergistic effects. For example, the administration of insulin with the noncompetitive NMDA antagonist, dizocilpine, in diabetic rats following ischemia resulted in additive neuroprotective effects.[78] However, testing combined administration of unproven drugs provides additional challenges for clinical trial design.[6]

Some stroke treatments are more appropriate at one time period in the evolution of stroke than at others. Three time periods will be considered here. The first is minutes to hours, the second is hours to days, and the third is days to months.

4.5.1 Post-Stroke (Time Frame of Minutes to Hours)

Neuroprotective therapies in the ischemic core will be helpful only if the blood supply to the ischemic brain can be reestablished. Hypoperfusion in the core and penumbra accounts for a greater proportion of the resulting injury than the subsequent degradative processes that occur in the penumbral region.[79] In addition to avoiding relative hypotension, the primary treatment for hypoperfusion is the use of interventions with the potential to restore flow, such as the use of a clot lysing drug (tPA, for example).

Other approaches include mechanical clot disruption and the use of suction devices, lasers, and ultrasound.[80] Although intravenous rt-PA remains the only FDA-approved thrombolytic drug therapy for stroke,[75] other drugs including both long-used and novel thrombolytics and glycoprotein IIb/IIIa receptor antagonists are being evaluated.[81] Even if the blood vessels can be reopened, there is a risk of hemorrhagic

infarction following reperfusion into ischemic areas. Thus, early reestablishment of blood supply is critical if possible.

4.5.2 Post-Stroke (Time Frame of Hours to Days)

4.5.2.1 Neuroimaging Techniques

Positron emission tomography (PET), diffusion–perfusion magnetic resonance imaging (MRI), and computerized tomography (CT) perfusion are now used to identify patients with potentially salvageable penumbral regions. In one study, the penumbral region accounted for 18% of the final infarct volume; the remaining 82% of the affected brain tissure was critically hypoperfused (70%) or sufficiently perfused (12%).[79] PET, the "gold standard" is not logistically feasible to guide urgent clinical treatment because it is not widely available and requires considerable set-up time. MRI techniques such as diffusion–perfusion weighted imaging, MR spectroscopy, and CT perfusion may prove more useful in detecting salvageable brain as part of routine clinical practice.[7,82] Because of person-to-person variations in collateral blood supplies, the use of neuroimaging may also allow the use of treatments that are not based solely on time since stroke onset.[82–84]

4.5.2.2 Receptor Antagonists, Calpain Inhibitors, and Free Radical Scavengers

Several other drugs intended to limit ischemic injury are being developed. The combination of NMDA antagonists with AMPA or kainate receptor antagonists may confer protection to oligodendrocytes and GABAergic neurons with Ca^{2+}-permeable AMPA receptors.[30] Toxicity can severely limit the clinical efficacy of otherwise useful treatment approaches. This applies to many drugs aimed at blocking excitotoxicity. Developing more targeted drugs may limit side effects. For example, ifenprodil acts on NR2B-containing NMDA receptors and they are expressed in greater proportions in the forebrain compared to the hindbrain.[30,85] Therefore, it is anticipated that its psychometric side effects might be reduced as compared to other drugs of this class.

Calpains are also receiving attention because they are proteolytic enzymes activated by calcium and may be potential targets for therapeutic agents. Calpains are activated following ischemia and break down cytoskeletal proteins such as spectrin. Calpain inhibitors including AK275, AK295, and MDL 28,170 are neuroprotective following ischemia in rats.[86–88] MDL 28,170 reduced infarct volume when administreed up to 6 hours following MCA occlusion.[88]

A number of potential therapeutic agents have been developed to reduce reperfusion-related injuries that involve the accumulation of oxygen free-radicals and inflammatory cells. The agents include superoxide dismutase, catalase, glutathione, iron chelators, vitamin E, alphaphenyl nitrogen (PBN), dimethylthiourea, oxypurinol, and tirilazad mesylate. They may act by reducing cytotoxic and vasogenic brain edema, aiding in Ca^{2+} homeostasis reestablishment, and antagonizing glutamate excitotoxicity.[80] Some have been tested in clinical trials, but none has yet proven efficacious.

One of the consequences of oxygen free radical formation is the destruction of single-strand DNA. This leads to the activation of poly (ADP-ribose) polymerase (PARP), a repair enzyme that depletes cellular nicotinamide adenine dinucleotide (NAD^+) and ATP.[89] Studies that eliminated the PARP gene or administered PARP inhibitors showed reduced infarction following ischemia.[89] However, the PARP enzyme may be important in DNA repair and genomic stability, particularly after partial DNA disruption from ischemia. It has also been hypothesized that because PARP activation involves NAD^+ that then depletes the metabolic pool of NADH, enhancing the pool of NAD^+ may contribute to enhanced cell functioning. Several papers have suggested that direct nicotinamide treatment may be effective at repleting the pool of metabolic NADH and also facilitating the repair processes of PARP.

4.5.2.3 Anti-Apoptosis/Necrosis Agents

Drug therapies (cycloheximide and anisomycin) have also been developed to inhibit apoptosis and have demonstrated neuroprotection in focal and global models of ischemia.[90,91] Thought to act through this mechanism, a caspase-3 inhibitor [N-benzyloxycarbonyl-Asp(Ome)-Glu(Ome)-Val-Asp(Ome)-fluoromethylketone or z-DEVD.FMK] reduced infarct size following transient ischemia.[11]

Neuronal apoptosis inhibitor protein (NAIP), a novel anti-apoptotic gene, is a group II (3 and 7) caspase inhibitor that may be able to reduce apoptosis.[92] Other inhibitors such as *N*-benzyloxycarbonyl-Val-Ala-Asp-fluoromethyl ketone (Z-VAD.FMK) and *N*-benzyloxycarbonyl-Asp-Glu-Val-Asp-fluoromethyl ketone (z-DEVD.FMK) that are not caspase selective and also block cathepsins reduce behavioral and cellular deficits as well as infarct volume following focal ischemia.[33,93,94]

A combination of anti-apoptotic and antinecrotic therapies may be advantageous. For example, the combined administration of dextrorphan and cycloheximide reduced infarct volume following transient ischemia (MCAO) in rats by 87%, which was greater than the reduction resulting from the use of either agent alone (~65%).[95] Another example of combination therapy is the use of MK-801 and z-VAD.fmk, which also reduced infarct size following ischemia.[96] It has been suggested, however, that if necrosis is reduced, apoptosis may become unmasked or promoted.[44]

4.5.2.4 Zinc Toxicity Treatment

Zinc toxicity following stroke is another potential area of therapeutic application. One consequence of zinc exposure is an increase in dihydroxy-acetone phosphate, a glycolytic intermediate, that in turn causes a decrease in neuronal ATP levels. It has been suggested that the administration of pyruvate, an energy substrate, can help ease the ATP loss. It has been postulated that the failure of calcium channel antagonists may in part be due to perturbations in zinc levels following ischemic injury. The reduction of zinc release from nerve terminals may be accomplished by a dietary restriction of zinc.[11] Other approaches to lessening the toxic effects of zinc could include the upregulation of both metallothioneins and cellular zinc extrusion transporters and the implementation of mechanisms that prevent energy metabolism interference.[30] Unfortunately, clinical trials targeting zinc have also failed.

4.5.2.5 Anti-Inflammatory Treatments

Neurons have been the primary targets of neuroprotective strategies. However, white matter and axons are also damaged following ischemia. Astrocytes may be injured as a result of the release of inflammatory mediators following ischemic insult as well as zinc toxicity.[97] Axons and oligodendrocytes are thought to incur damage as a consequence of calcium influx through the Na^+/Ca^{2+} exchanger and AMPA receptor over-stimulation, respectively.[98] The release of glutamate via reverse Na^+-glutamate transport may also contribute to oligodendrocyte damage.[65]

Potential therapeutic targets may involve interfering with various steps of the inflammatory cascades. For example, microvascular occlusion may be reduced by the inhibition of leukocyte adherence to blood vessels in the ischemic area. Other strategies include directing antibodies toward molecules such as intercellular adhesion molecule-1 (ICAM-1)[99] and inhibiting the release of proinflammatory cytokines from astrocytes and microglia such as interleukin-1β (IL-1β) or tumor necrosis factor-α (TNF-α). The use of statins and estrogens may also have the potential to reduce injury following ischemic insult through upregulation of endothelial nitric oxide synthase[100] and antioxidant and trophic mechanisms,[101] respectively.

The use of growth factors may also be beneficial in treating ischemic injury and promoting functional recovery. Exogenous compounds such as nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophins 4/5 (NT-4/5), basic fibroblast growth factor, and insulin-like growth factor-1 (IGF-1) can all reduce injuries in rats subjected to cerebral ischemia.[30] One clinical trial of fibroblast growth factor (FGF) was stopped because of toxicity.

The inflammatory response is initiated and regulated by the complement system that consists of a number of cascades. The complement system causes injury in animal models of ischemia through the production of anaphylotoxins C3a and C5a and endothelial cell adhesion molecule upregulation.[102] The complement cascade offers several sites of potential therapeutic intervention. For example, soluble complement receptor-1 (sCR1), a strong inhibitor of complement activation, reduced neurological deficits and decreased platelet and polymorphonuclear leukocytes (PMN) accumulations following MCAO and reperfusion in mice.[103]

Protein kinase C increases both the vesicular release of glutamate and neuronal excitability.[104] Pretreatment with agents such as staurosporine, a broad-spectrum protein kinase inhibitor[105] and 1-(5-isoquinolinesulfonyl)-2-methylpiperazine dihydrochloride (H-7)[106] decreased neuronal cell death following global cerebral ischemia and the accumulation of extracellular glutamate, respectively.

The mitogen-activated protein (MAP) kinase pathways may also be activated during ischemia. These include the c-Jun NH_2-terminal kinases (JNKs), p38 kinases, and extracellular signal-regulated kinases (ERKs). The p38 inhibitor, SB203580, administered as a pretreatment reduced neuronal death in a global model of ischemia.[107] However, following transient focal ischemia, it was not effective.[108] The PD98059 ERK inhibitor also given as a pretreatment decreased the volume of infarction following transient focal ischemia.[108]

4.5.2.6 Hypothermia

In addition to the plethora of pharmacological agents that may provide therapeutic benefits following stroke, physiological variables can be manipulated to confer protection. Hypothermia has been studied for the past 50 years because of its protective benefits[109] and has been used to protect organs during cardiovascular and neurosurgical procedures. In the case of stroke, reduced body temperatures in patients admitted to hospitals resulted in both lower mortality rates and improved functional outcomes.[110,111] Among the potential mechanisms by which hypothermia offers protection are: reduction in metabolic rate, thereby delaying the depletion of high-energy phosphates, inhibition of excitatory neurotransmitter release and oxygen radical production, decrease in intracranial pressure and anti-convulsant activity, and suppression of initiation of spreading depression (see Clifton et al. and Bernard and Buist[112,113] for references).

Temperatures of 32 to 34°C have been demonstrated in animal models to be safe and produce a minimum number of side effects.[112,114,115] In patients who suffer hypothermic side effects such as platelet dysfunction, rebound hyperthermia, and pneumonia,[116] a combination of more modest reductions in temperature (2 to 3°C) with neuroprotective drug therapies may be more effective. The efficacy of hypothermia seems to depend on the length of its application following ischemic injury. Hippocampal CA1 cells are protected when the duration of hypothermia is increased from 12 to 24 hours.[117–119] The time window between the onset of ischemia and irreversible cell injury increases as the duration of hypothermia is increased.[120] Preliminary clinical trials of hypothermia are promising.[121,122]

In contrast to hypothermia, any increases in brain temperature above normal (37°C) following stroke can exacerbate ischemic injury.[123,124] Hyperthermia in stroke patients is associated with increases in morbidity and mortality rates.[125,126] Hyperthermia has also been shown to interfere with the actions of therapeutic agents such as MK-801 and thrombolytic treatments.[127] Fevers must be treated aggressively in patients with ischemic stroke.[7]

4.5.2.7 Hyperglycemia Treatment

Hyperglycemia, another physiological variable that can be manipulated in the clinical environment, has been associated with poor outcomes following strokes in animal studies and clinical trials. The multicenter Trial of ORG 10172 in Acute Stroke Treatment (TOAST) found that higher blood glucose levels resulted in worse outcomes (odds ratio: 0.82 for every 100 mg/dL increase in glucose; $p = 0.03$).[128] Hyperglycemia increases cerebral lactate concentrations, causes neuronal and glial damage, and increases infarct volume.[129–131] Pre-ischemic hyperglycemia also increases extracellular glutamate concentrations during ischemia, which results in exacerbated cell damage in the neocortex.[132] In contrast, relative hypoglycemia in the presence of permanent focal ischemia results in a smaller infarct volume as compared to severe hyperglycemic conditions.[130] Insulin was neuroprotective in a number of animal studies following global and focal ischemia (see Kagansky et al.[133] for references). IGF-1 has also demonstrated neuroprotective properties.[134]

The Glucose Insulin in Stroke Trial is examining the potential protective effects of the combined administration of glucose, potassium, and insulin (GKI) in stroke patients with mild to moderate hyperglycemia. Results from the pilot study indicate a slightly lower mortality rate in GKI patients compared to controls (28 versus 32%).* During the first 24 hours of hospitalization following stroke, hyperglycemia should be avoided by excluding the administration of dextrose-containing solutions. By consensus, the upper limit of glucose concentration range in all patients should be maintained at ≤300 mg/dL.[7]

4.5.3 Post-Stroke (Time Frame of Days to Months)

A number of therapeutic approaches can be employed in the days or months after stroke.[49] In addition to drugs aimed at secondary prevention, orally active drugs may eventually be developed to confer long-lasting neuroprotection in persons at risk for recurrent stroke.[69] Pharmacological strategies designed to facilitate the recovery process are also under investigation. For example, amphetamine enhances sensory and motor function following ischemia.[135] Other drugs such as yohimbine,[136] phenylpropanolamine,[137] and methylphenidate[138] enhance motor recovery following brain injury as a result of their effects on norepinephrine.

However, drugs that decrease norepinephrine release such as clonidine hydrochloride (α2-adrenergic receptor agonist), prazosin, and phenoxybenzamine (α1-adrenergic receptor antagonists) interfere with motor recovery following brain injury.[139] Therefore, the use of certain drugs given for nonstroke morbidities should be avoided because they may interfere with long-term stroke outcomes.[139]

Other novel approaches aimed at improving post-stroke recovery include stem cell transplantation and gene therapy (see Chapter 2). In rat models of stroke, the transplantation of cultured neuronal cells improved motor and cognitive deficits and was safe.[140,141] An initial trial was conducted in humans. Cultured neurons (human precursor cell lines differentiated into neurons) were injected into the area of infarction. No major adverse consequences appeared as long as 12 to 18 months following transplantation, but clinical benefit remains uncertain.[142]

Physiotherapeutic approaches are central in reducing mortality and improving long-term outcomes. Patient mobilization can also help to reduce the occurrence of pneumonia and secondary thromboembolic events.[143] A variety of new physiotherapeutic approaches are under investigation including constraint-induced therapy, robot-assistive training, and supported treadmill training.[144]

4.5.4 Surgical Treatment Options

Although this discussion has focused on medical interventions, surgical treatments are also being explored for stroke treatment. For example, hemicraniectomy (removal of the skull and dura on one side of the head for decompression of the brain) may be useful in patients at risk for herniation after nondominant hemisphere stroke.[121] Intensive care management of patients with acute ischemic stroke is also evolving (see Chapter 13). For example, the potential impact of monitoring physiological

* Internet Stroke Center: www.strokecenter.org.

parameters such as brain tissue oxygenation are being explored, together with aggressive hyperdynamic therapy to enhance blood flow into ischemic regions and collateral formation.

4.6 CONCLUSIONS

Stroke remains one of the leading causes of death and disability worldwide. Attempts to develop effective drug therapies for stroke-related brain damage have been fraught with difficulties. A number of issues must be addressed before successful results from preclinical studies can be translated to the treatment of stroke patients. Valuable lessons learned from past failures can be used to increase the chances of producing efficacious drug therapies for stroke. Many promising avenues of stroke treatment remain, but enhancing their delivery to the vascular-compromised brain remains a further challenge for the future.

REFERENCES

1. Bonita, R. and Beaglehole, R., Monitoring stroke: an international challenge, *Stroke*, 26 (4), 541, 1995.
2. American Heart Association, *Heart Disease and Stroke Statistics: 2003 Update*, Dallas, TX, 2003.
3. Bonita, R., Epidemiology of stroke [comment], *Lancet*, 339 (8789), 342, 1992.
4. Kidwell, C.S. et al., Trends in acute ischemic stroke trials through the 20th century, *Stroke*, 32 (6), 1349, 2001.
5. Finklestein, S.P. et al., Stroke Therapy Academic Industry Roundtable I: recommendations for standards regarding preclinical neuroprotective and restorative drug development, *Stroke*, 30 (12), 2752, 1999.
6. Albers, G.W. et al., Stroke Therapy Academic Industry Roundtable II: recommendations for clinical trial evaluation of acute stroke therapies [comment], *Stroke*, 32 (7), 1598, 2001.
7. Adams, H.P., Jr. et al., Guidelines for the early management of patients with ischemic stroke: scientific statement from the Stroke Council of the American Stroke Association, *Stroke,* 34 (4), 1056, 2003.
8. Bogousslavsky, J. and Regli, F., Cerebral infarction with transient signs (CITS): do TIAs correspond to small deep infarcts in internal carotid artery occlusion? *Stroke,* 15 (3), 536, 1984.
9. Mayberg, M.R. et al., Guidelines for the management of aneurysmal subarachnoid hemorrhage: a statement for healthcare professionals from a special writing group of the Stroke Council, American Heart Association, *Stroke,* 25 (11), 2315, 1994.
10. Zivin, J.A., Factors determining the therapeutic window for stroke, *Neurology*, 50 (3), 599, 1998.
11. Lee, J.M. et al., Brain tissue responses to ischemia, *Journal of Clinical Investigations*, 106 (6), 723, 2000.
12. Choi, D.W., Glutamate neurotoxicity and diseases of the nervous system, *Neuron*, 1 (8), 623, 1988.
13. Rothwell, N.J. and Hopkins, S.J., Cytokines and the nervous system II: actions and mechanisms of action [comment], *Trends in Neurosciences*, 18 (3), 130, 1995.

14. Kristian, T. and Siesjo, B.K., Calcium–related damage in ischemia, *Life Sciences*, 59 (5–6), 357, 1996.
15. Martin, R.L., Lloyd, H.G., and Cowan, A.I., The early events of oxygen and glucose deprivation: setting the scene for neuronal death? *Trends in Neurosciences*, 17 (6), 251, 1994.
16. Katsura, K., Kristian, T., and Siesjo, B.K., Energy metabolism, ion homeostasis, and cell damage in the brain, *Biochemical Society Transactions*, 22 (4), 991, 1994.
17. Choi, D.W., Calcium-mediated neurotoxicity: relationship to specific channel types and role in ischemic damage, *Trends in Neurosciences*, 11 (10), 465, 1988.
18. Choi, D.W., Calcium: still center-stage in hypoxic–ischemic neuronal death, *Trends in Neurosciences,* 18 (2), 58, 1995.
19. Hossmann, K.A., Peri-infarct depolarizations, *Cerebrovascular and Brain Metabolism Reviews*, 8 (3), 195, 1996.
20. Mies, G., Iijima, T., and Hossmann, K.A., Correlation between peri-infarct DC shifts and ischaemic neuronal damage in rat, *Neuroreport*, 4 (6), 709, 1993.
21. Zhang, Y. et al., The oxidative inactivation of mitochondrial electron transport chain components and ATPase, *Journal of Biological Chemistry*, 265 (27), 16330, 1990.
22. Matsumoto, S. et al., Blockade of the mitochondrial permeability transition pore diminishes infarct size in the rat after transient middle cerebral artery occlusion, *Journal of Cerebral Blood Flow and Metabolism*, 19 (7), 736, 1999.
23. Hossmann, K.A., Viability thresholds and the penumbra of focal ischemia [comment], *Annals of Neurology*, 36 (4), 557, 1994.
24. Kato, H. and Kogure, K., Biochemical and molecular characteristics of the brain with developing cerebral infarction, *Cellular and Molecular Neurobiology.* 19 (1), 93, 1999. Erratum in *Cellular and Molecular Neurobiology*, 20 (3), 417, 2000.
25. Kuroda, S. and Siesjo, B.K., Reperfusion damage following focal ischemia: pathophysiology and therapeutic windows, *Clinical Neuroscience*, 4 (4), 199, 1997.
26. Dirnagl, U., Iadecola, C., and Moskowitz, M.A., Pathobiology of ischaemic stroke: an integrated view, *Trends in Neurosciences,* 22 (9), 391, 1999.
27. Martin, L.J. et al., Neurodegeneration in excitotoxicity, global cerebral ischemia, and target deprivation: perspective on the contributions of apoptosis and necrosis, *Brain Research Bulletin*, 46 (4), 281, 1998.
28. Choi, D.W., Ischemia-induced neuronal apoptosis, *Current Opinions in Neurobiology*, 6 (5), 667, 1996.
29. Fujimura, M. et al., Cytosolic redistribution of cytochrome C after transient focal cerebral ischemia in rats, *Journal of Cerebral Blood Flow and Metabolism*, 18 (11), 1239, 1998.
30. Lee, J.M., Zipfel, G.J., and Choi, D.W., The changing landscape of ischaemic brain injury mechanisms, *Nature*, 399 (6738; Suppl.), A7, 1999.
31. Degraba, T.J., The role of inflammation after acute stroke: utility of pursuing anti-adhesion molecule therapy, *Neurology*, 51 (Suppl, 3), S62, 1998.
32. Hallenbeck, J.M., Inflammatory reactions at the blood–endothelial interface in acute stroke, *Advances in Neurology*, 71, 281, 1996.
33. Hara, H. et al., Inhibition of interleukin 1-beta converting enzyme family proteases reduces ischemic and excitotoxic neuronal damage, *Proceedings of the National Academy of Sciences of the United States of America*, 94 (5), 2007, 1997.
34. Sensi, S.L. et al., Measurement of intracellular free zinc in living cortical neurons: routes of entry, *Journal of Neuroscience*, 17 (24), 9554, 1997.
35. Sorensen, J.C. et al., Rapid disappearance of zinc positive terminals in focal brain ischemia, *Brain Research*, 812 (1–2), 265, 1998.

36. Astrup, J., Siesjo, B.K., and Symon, L., Thresholds in cerebral ischemia: the ischemic penumbra, *Stroke,* 12 (6), 723, 1981.
37. Pulsinelli, W., Pathophysiology of acute ischaemic stroke, *Lancet*, 339 (8792), 533, 1992.
38. Grotta, J., Rodent models of stroke limitations: what can we learn from recent clinical trials of thrombolysis? [comment], *Archives of Neurology*, 53 (10), 1067, 1996.
39. Ginsberg, M.D., The concept of the therapeutic window: a synthesis of critical issues, in *Nineteenth Princeton Stroke Conference*, Moskowitz, M.A. and Caplan, L.R., Eds., Butterworth–Heinemann, Boston, 1995, p. 331.
40. Heiss, W.D. et al., Progressive derangement of peri-infarct viable tissue in ischemic stroke, *Journal of Cerebral Blood Flow and Metabolism*, 12 (2), 193, 1992.
41. Rothman, S.M. and Olney, J.W., Glutamate and the pathophysiology of hypoxic–ischemic brain damage, *Annals of Neurology*, 19 (2), 105, 1986.
42. Martinez-Vila, E. and Sieira, P.I., Current status and perspectives of neuroprotection in ischemic stroke treatment, *Cerebrovascular Diseases*, 11 (Suppl. 1), 60, 2001.
43. Fisher, M. and Schaebitz, W., An overview of acute stroke therapy: past, present, and future, *Archives of Internal Medicine*, 160 (21), 3196, 2000.
44. Gladstone, D.J. et al., Toward wisdom from failure: lessons from neuroprotective stroke trials and new therapeutic directions, *Stroke,* 33 (8), 2123, 2002.
45. Hossmann, K.A., Animal models of cerebral ischemia 1: review of literature, *Cerebrovascular Diseases*, 1, 2, 1991.
46. Mohr, J.P. and Barnett, H.J.M., Classification of ischemic strokes, in *Stroke: Pathophysiology, Diagnosis, and Management*, Barnett, H.J.M. et al., Eds., Churchill Livingstone, New York, 1986, p. 281.
47. Lightfoote, W.E., II, Molinari, G.F., and Chase, T.N., Modification of cerebral ischemic damage by anesthetics, *Stroke,* 8 (5), 627, 1977.
48. Ginsberg, M.D., The validity of rodent brain ischemia models is self-evident [comment], *Archives of Neurology*, 53 (10), 1065, 1996.
49. Adams, H.P., Jr., Treating ischemic stroke as an emergency, *Archives of Neurology*, 55 (4), 457, 1998.
50. Fisher, M., Characterizing the target of acute stroke therapy, *Stroke,* 28 (4), 866, 1997.
51. Kuroda, S. et al., Neuroprotective effects of a novel nitrone, NXY-059, after transient focal cerebral ischemia in the rat, *Journal of Cerebral Blood Flow and Metabolism*, 19 (7), 778, 1999.
52. Sydserff, S.G. et al., Effect of NXY-059 on infarct volume after transient or permanent middle cerebral artery occlusion in the rat: studies on dose, plasma concentration and therapeutic time window, *British Journal of Pharmacology*, 135 (1), 103, 2002.
53. Drummond, J.C., Piyash, P.M., and Kimbro, J.R., Neuroprotection failure in stroke [comment], *Lancet*, 356 (9234), 1032, 2000.
54. Valtysson, J. et al., Neuropathological endpoints in experimental stroke pharmacotherapy: the importance of both early and late evaluation, *Acta Neurochirurgica*, 129 (1–2), 58, 1994.
55. Colbourne, F. et al., Continuing postischemic neuronal death in CA1: influence of ischemia duration and cytoprotective doses of NBQX and SNX-111 in rats, *Stroke,* 30 (3), 662, 1999.
56. Wang, F. et al., Inhibition of cyclin-dependent kinases improves CA1 neuronal survival and behavioral performance after global ischemia in the rat, *Journal of Cerebral Blood Flow and Metabolism*, 22 (2), 171, 2002.

57. Duncan, P.W., Jorgensen, H.S., and Wade, D.T., Outcome measures in acute stroke trials: a systematic review and some recommendations to improve practice [comment], *Stroke,* 31 (6), 1429, 2000.
58. Corbett, D. and Nurse, S., The problem of assessing effective neuroprotection in experimental cerebral ischemia, *Progress in Neurobiology*, 54 (5), 531, 1998.
59. Kawamata, T. et al., Intracisternal basic fibroblast growth factor (bFGF) enhances behavioral recovery following focal cerebral infarction in the rat, *Journal of Cerebral Blood Flow and Metabolism*, 16 (4), 542, 1996.
60. Kawamata, T. et al., Intracisternal osteogenic protein-1 enhances functional recovery following focal stroke, *Neuroreport*, 9 (7), 1441, 1998.
61. Hunter, A.J., Mackay, K.B., and Rogers, D.C., To what extent have functional studies of ischaemia in animals been useful in the assessment of potential neuroprotective agents? *Trends in Pharmacological Sciences*, 19 (2), 59, 1998.
62. Hudzik, T.J. et al., Long-term functional end points following middle cerebral artery occlusion in the rat, *Pharmacology, Biochemistry and Behavior*, 65 (3), 553, 2000.
63. Lyden, P.D. et al., Quantitative effects of cerebral infarction on spatial learning in rats, *Experimental Neurology*, 116 (2), 122, 1992.
64. Grotta, J.C. et al., CGS-19755, a competitive NMDA receptor antagonist, reduces calcium-calmodulin binding and improves outcome after global cerebral ischemia, *Annals of Neurology*, 27 (6), 612, 1990.
65. Stys, P.K., Anoxic and ischemic injury of myelinated axons in CNS white matter: from mechanistic concepts to therapeutics, *Journal of Cerebral Blood Flow and Metabolism*, 18 (1), 2, 1998.
66. Muir, K.W. and Grosset, D.G., Neuroprotection for acute stroke: making clinical trials work, *Stroke,* 30 (1), 180, 1999.
67. Small, D.L. and Buchan, A.M., Animal models, *British Medical Bulletin*, 56 (2), 307, 2000.
68. Pardridge, W.M., Why is the global CNS pharmaceutical market so under-penetrated? *Drug Discovery Today*, 7 (1), 5, 2002.
69. Dyker, A.G. and Lees, K.R., Duration of neuroprotective treatment for ischemic stroke, *Stroke,* 29 (2), 535, 1998.
70. Coimbra, C. et al., Long-lasting neuroprotective effect of postischemic hypothermia and treatment with an anti-inflammatory/antipyretic drug: evidence for chronic encephalopathic processes following ischemia, *Stroke*, 27 (9), 1578, 1996.
71. Muir, K.W. and Lees, K.R., Clinical experience with excitatory amino acid antagonist drugs, *Stroke*, 26 (3), 503, 1995.
72. Lees, K.R., Advances in neuroprotection trials, *European Neurology*, 45 (1), 6, 2001.
73. Degraba, T.J. and Pettigrew, L.C., Why do neuroprotective drugs work in animals but not humans? *Neurologic Clinics*, 18 (2), 475, 2000.
74. Tilley, B.C. et al., Use of a global test for multiple outcomes in stroke trials with application to the National Institute of Neurological Disorders and Stroke t-PA Stroke Trial, *Stroke,* 27 (11), 2136, 1996.
75. National Institute of Neurological Disorders and Stroke rt-PA Stroke Study Group, Tissue plasminogen activator for acute ischemic stroke [comment], *New England Journal of Medicine*, 333 (24), 1581, 1995.
76. Fisher, M., Recommendations for advancing development of acute stroke therapies: Stroke Therapy Academic Industry Roundtable 3, *Stroke,* 34 (6), 1539, 2003.
77. Storer, B.E., A sequential Phase II/III trial for binary outcomes, *Statistics in Medicine*, 9, 229, 1990.

78. Bomont, L. and Mackenzie, E.T., Neuroprotection after focal cerebral ischaemia in hyperglycaemic and diabetic rats, *Neuroscience Letter*, 197 (1), 53, 1995.
79. Heiss, W.D. et al., Which targets are relevant for therapy of acute ischemic stroke? [comment], *Stroke,* 30 (7), 1486, 1999.
80. Lindsberg, P.J. et al., The future of stroke treatment, *Neurologic Clinics*, 18 (2), 495, 2000.
81. Verheugt, F.W., Acute coronary syndromes: drug treatments, *Lancet*, 353 (Suppl. 2), SII20, 1999.
82. Baird, A.E. and Warach, S., Magnetic resonance imaging of acute stroke, *Journal of Cerebral Blood Flow and Metabolism*, 18 (6), 583, 1998. Erratum in *Journal of Cerebral Blood Flow and Metabolism*, 18 (10), preceding 19047, 1998.
83. Barber, P.A. et al., Absent middle cerebral artery flow predicts the presence and evolution of the ischemic penumbra, *Neurology*, 52 (6), 1125, 1999.
84. Nabavi, D.G. et al., Perfusion mapping using computed tomography allows accurate prediction of cerebral infarction in experimental brain ischemia, *Stroke,* 32 (1), 175, 2001.
85. Portera-Cailliau, C., Price, D.L., and Martin, L.J., N-methyl-D-aspartate receptor proteins NR2A and NR2B are differentially distributed in the developing rat central nervous system as revealed by subunit-specific antibodies, *Journal of Neurochemistry*, 66 (2), 692, 1996.
86. Bartus, R.T. et al., Calpain inhibitor AK295 protects neurons from focal brain ischemia: effects of post-occlusion intra-arterial administration, *Stroke,* 25 (11), 2265, 1994.
87. Bartus, R.T. et al., Postischemic administration of AK275, a calpain inhibitor, provides substantial protection against focal ischemic brain damage, *Journal of Cerebral Blood Flow and Metabolism*, 14 (4), 537, 1994.
88. Markgraf, C.G. et al., Six-hour window of opportunity for calpain inhibition in focal cerebral ischemia in rats, *Stroke,* 29 (1), 152, 1998.
89. Szabo, C. and Dawson, V.L., Role of poly(ADP-ribose) synthetase in inflammation and ischaemia-reperfusion, *Trends in Pharmacological Sciences*, 19 (7), 287, 1998.
90. Goto, K. et al., Effects of cycloheximide on delayed neuronal death in rat hippocampus, *Brain Research*, 534 (1–2), 299, 1990.
91. Shigeno, T. et al., Reduction of delayed neuronal death by inhibition of protein synthesis, *Neuroscience Letter,* 120 (1), 117, 1990.
92. Robertson, G.S. et al., Neuroprotection by the inhibition of apoptosis, *Brain Pathology*, 10 (2), 283, 2000.
93. Endres, M. et al., Attenuation of delayed neuronal death after mild focal ischemia in mice by inhibition of the caspase family, *Journal of Cerebral Blood Flow and Metabolism*, 18 (3), 238, 1998.
94. Schotte, P. et al., Non–specific effects of methyl ketone peptide inhibitors of caspases, *FEBS Letters*, 442 (1), 117, 1999.
95. Du, C. et al., Additive neuroprotective effects of dextrorphan and cycloheximide in rats subjected to transient focal cerebral ischemia, *Brain Research*, 718 (1–2), 233, 1996.
96. Ma, J., Endres, M., and Moskowitz, M.A., Synergistic effects of caspase inhibitors and MK-801 in brain injury after transient focal cerebral ischaemia in mice, *British Journal of Pharmacology*, 124 (4), 756, 1998.
97. Choi, D.W. and Koh, J.Y., Zinc and brain injury, *Annual Review of Neuroscience*, 21, 347, 1998.

98. Waxman, S.G. et al., Anoxic injury of rat optic nerve: ultrastructural evidence for coupling between Na^+ influx and Ca^{2+}-mediated injury in myelinated CNS axons, *Brain Research*, 644 (2), 197, 1994.
99. Zhang, R.L. et al., Anti-ICAM-1 antibody reduces ischemic cell damage after transient middle cerebral artery occlusion in the rat, *Neurology*, 44 (9), 1747, 1994.
100. Endres, M. et al., Stroke protection by 3-hydroxy-3-methylglutaryl (HMG)-CoA reductase inhibitors mediated by endothelial nitric oxide synthase, *Proceedings of the National Academy of Sciences of the United States of America*, 95 (15), 8880, 1998.
101. Toung, T.J., Traystman, R.J., and Hurn, P.D., Estrogen-mediated neuroprotection after experimental stroke in male rats, *Stroke,* 29 (8), 1666, 1998.
102. De Keyser, J., Sulter, G., and Luiten, P.G., Clinical trials with neuroprotective drugs in acute ischaemic stroke: are we doing the right thing? [comment], *Trends in Neurosciences,* 22 (12), 535, 1999.
103. Huang, J. et al., Neuronal protection in stroke by an sLex-glycosylated complement inhibitory protein, *Science*, 285 (5427), 595, 1999.
104. Kaczmarek, L.K., The role of protein kinase C in the regulation of ion channels and neurotransmitter release, *Trends in Neurosciences,* 10, 30, 1987.
105. Hara, H. et al., Staurosporine, a novel protein kinase C inhibitor, prevents postischemic neuronal damage in the gerbil and rat, *Journal of Cerebral Blood Flow and Metabolism*, 10 (5), 646, 1990.
106. Nakane, H. et al., Protein kinase C modulates ischemia-induced amino acids release in the striatum of hypertensive rats, *Brain Research*, 782 (1–2), 290, 1998.
107. Sugino, T., Nozaki, K., and Hashimoto, N., Activation of mitogen-activated protein kinases in gerbil hippocampus with ischemic tolerance induced by 3-nitropropionic acid, *Neuroscience Letters*, 278 (1–2), 101, 2000.
108. Alessandrini, A. et al., MEK1 protein kinase inhibition protects against damage resulting from focal cerebral ischemia, *Proceedings of the National Academy of Sciences of the United States of America*, 96 (22), 12866, 1999.
109. Bigelow, W.G., Lindsay, W.K., and Greenwood, W.F., Hypothermia: its possible role in cardiac surgery, *Annals of Surgery*, 132, 849, 1950.
110. Reith, J. et al., Body temperature in acute stroke: relation to stroke severity, infarct size, mortality, and outcome [comment], *Lancet*, 347 (8999), 422, 1996.
111. Jorgensen, H.S. et al., What determines good recovery in patients with the most severe strokes? The Copenhagen Stroke Study, *Stroke,* 30 (10), 2008, 1999.
112. Clifton, G.L. et al., Systemic hypothermia in treatment of brain injury, *Journal of Neurotrauma*, 9 (Suppl. 2), S487, 1992.
113. Bernard, S.A. and Buist, M., Induced hypothermia in critical care medicine: a review, *Critical Care Medicine*, 31 (7), 2041, 2003.
114. Marion, D.W. et al., The use of moderate therapeutic hypothermia for patients with severe head injuries: a preliminary report [comment], *Journal of Neurosurgery*, 79 (3), 354, 1993.
115. Piepgras, A. et al., Rapid active internal core cooling for induction of moderate hypothermia in head injury by use of an extracorporeal heat exchanger, *Neurosurgery*, 42 (2), 311, 1998.
116. Sessler, D.I., Mild perioperative hypothermia, *New England Journal of Medicine*, 336 (24), 1730, 1997.
117. Colbourne, F. and Corbett, D., Delayed and prolonged post-ischemic hypothermia is neuroprotective in the gerbil, *Brain Research*, 654 (2), 265, 1994.

118. Colbourne, F. and Corbett, D., Delayed postischemic hypothermia: a six-month survival study using behavioral and histological assessments of neuroprotection, *Journal of Neuroscience*, 15 (11), 7250, 1995.
119. Colbourne, F., Sutherland, G., and Corbett, D., Postischemic hypothermia: a critical appraisal with implications for clinical treatment, *Molecular Neurobiology*, 14 (3), 171, 1997.
120. Corbett, D. and Thornhill, J., Temperature modulation (hypothermic and hyperthermic conditions) and its influence on histological and behavioral outcomes following cerebral ischemia, *Brain Pathology*, 10 (1), 145, 2000.
121. Schwab, S. et al., Early hemicraniectomy in patients with complete middle cerebral artery infarction, *Stroke,* 29 (9), 1888, 1998.
122. Schwab, S. et al., Feasibility and safety of moderate hypothermia after massive hemispheric infarction [comment], *Stroke,* 32 (9), 2033, 2001.
123. Busto, R. et al., Small differences in intraischemic brain temperature critically determine the extent of ischemic neuronal injury, *Journal of Cerebral Blood Flow and Metabolism*, 7 (6), 729, 1987.
124. Morikawa, E. et al., The significance of brain temperature in focal cerebral ischemia: histopathological consequences of middle cerebral artery occlusion in the rat, *Journal of Cerebral Blood Flow and Metabolism*, 12 (3), 380, 1992.
125. Hindfelt, B., The prognostic significance of subfebrility and fever in ischaemic cerebral infarction, *Acta Neurologica Scandinavica*, 53 (1), 72, 1976.
126. Castillo, J. et al., Timing for fever-related brain damage in acute ischemic stroke [comment], *Stroke,* 29 (12), 2455, 1998.
127. Memezawa, H. et al., Hyperthermia nullifies the ameliorating effect of dizocilpine maleate (MK-801) in focal cerebral ischemia, *Brain Research*, 670 (1), 48, 1995.
128. Bruno, A. et al., Acute blood glucose level and outcome from ischemic stroke: Trial of ORG 10172 in Acute Stroke Treatment (TOAST) Investigators, *Neurology*, 52 (2), 280, 1999.
129. Siesjo, B.K. et al., Molecular mechanisms of acidosis-mediated damage, *Acta Neurochirurgica Supplementum*, 66, 8, 1996.
130. Anderson, R.E. et al., Effects of glucose and PaO2 modulation on cortical intracellular acidosis, NADH redox state, and infarction in the ischemic penumbra, *Stroke,* 30 (1), 160, 1999.
131. Hoxworth, J.M. et al., Cerebral metabolic profile, selective neuron loss, and survival of acute and chronic hyperglycemic rats following cardiac arrest and resuscitation, *Brain Research*, 821 (2), 467, 1999.
132. Li, P.A. et al., Hyperglycemia enhances extracellular glutamate accumulation in rats subjected to forebrain ischemia [comment], *Stroke,* 31 (1), 183, 2000.
133. Kagansky, N., Levy, S., and Knobler, H., The role of hyperglycemia in acute stroke, *Archives of Neurology*, 58 (8), 1209, 2001.
134. Zhu, C.Z. and Auer, R.N., Intraventricular administration of insulin and IGF-1 in transient forebrain ischemia, *Journal of Cerebral Blood Flow and Metabolism*, 14 (2), 237, 1994.
135. Aichner, F., Adelwohrer, C., and Haring, H.P., Rehabilitation approaches to stroke, *Journal of Neural Transmission Supplementum*, (63), 59, 2002.
136. Feeney, D.M. and Westerberg, V.S., Norepinephrine and brain damage: alpha noradrenergic pharmacology alters functional recovery after cortical trauma, *Canadian Journal of Psychology*, 44 (2), 233, 1990.
137. Feeney, D.M. and Sutton, R.L., Pharmacotherapy for recovery of function after brain injury, *Critical Reviews in Neurobiology*, 3 (2), 135, 1987.

138. Kline, A.E. et al., Methylphenidate treatment following ablation-induced hemiplegia in rat: experience during drug action alters effects on recovery of function, *Pharmacology, Biochemistry and Behavior*, 48 (3), 773, 1994.
139. Goldstein, L.B., Potential effects of common drugs on stroke recovery, *Archives of Neurology*, 55 (4), 454, 1998.
140. Kleppner, S.R. et al., Transplanted human neurons derived from a teratocarcinoma cell line (NTera-2) mature, integrate, and survive for over one year in the nude mouse brain, *Journal of Comparative Neurology*, 357 (4), 618, 1995.
141. Borlongan, C.V. et al., Transplantation of cryopreserved human embryonal carcinoma-derived neurons (NT2N cells) promotes functional recovery in ischemic rats, *Experimental Neurology*, 149 (2), 310, 1998.
142. Kondziolka, D. et al., Transplantation of cultured human neuronal cells for patients with stroke [comment], *Neurology*, 55 (4), 565, 2000.
143. Johansson, B.B., Brain plasticity and stroke rehabilitation: the Willis lecture, *Stroke,* 31 (1), 223, 2000.
144. Goldstein, L.B., New approaches to poststroke rehabilitation, in *Pharmacology of Cerebral Ischemia*, Krieglstein, J. and Klumpp, S., Eds., MedPharm, Germany, 2002, p. 487.