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CURRENT AND EXPERIMENTAL TREATMENT OF STROKE

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Stroke is the third leading cause of death in the United States; approximately 730,000 Americans have a new or recurrent stroke each year. That's one every minute and it costs the health care system $30 billion annually. The incidence of stroke is expected to rise dramatically as the population ages because stroke risk increases with age. The risk of stroke doubles for each decade after age 55. Stroke is a major factor in the late-life dementia that affects more than 40% of Americans over the age of 80. One in four men will have had a disabling stroke by the age of 80 and one in five women by the age of 85.

Currently Alteplase (recombinant tissue plasminogen activator [rtPA]) is the only treatment for acute stroke approved in the United States. Alteplase is a thrombolytic agent that restores cerebral blood flow by removing the vascular occlusion. Alteplase is only an appropriate therapy for a small proportion of stroke patients (~2%) because it must be given early to achieve efficacy and functional recovery following delayed reperfusion. Less than 15% of patients, however, are admitted to the hospital within the 3-hour safety window (1). Several other new treatments are being tested in the clinic and even more are in preclinical development. Antiplatelet therapy and thrombolytics are aimed at improving cerebral blood flow but there are other therapeutic strategies such as neuroprotectants, antiinflammatory agents, free radical scavengers, and neurotrophic agents. In this chapter we survey the current status of clinical trials for stroke and review these therapeutic strategies.

PATHOPHYSIOLOGY AND PRECLINICAL MODELS OF STROKE

Stroke in the clinic is represented predominantly by ischemic stroke (80%), in which there is a loss of cerebral blood flow owing to vascular occlusion. The remaining 20% of strokes result from cerebral hemorrhage. Experimentally, there are two main types of *in vivo* stroke models, global and focal ischemia. In global models, the entire forebrain is made ischemic for a brief period (5 to 20 minutes) and then reperfused. After a significant delay (>3 days), selective neuronal death evolves in layers III, V, and VI of the cortex, the CA1 region of the hippocampus, and the striatum (2–4). These models are representative of cardiac arrest and coronary artery bypass surgery rather than clinical stroke. In focal models, the middle cerebral artery is occluded for a more prolonged period (60 to 120 minutes in temporary models and permanently in permanent occlusion models). Focal models are representative of clinical stroke and produce histologic damage similar to ischemic stroke in humans. The focal model produces a pannecrotic core surrounded by a narrow penumbral border. The penumbra is at risk of becoming necrotic but is potentially salvageable given the appropriate therapeutic intervention (3,4). The evolution of delayed neuronal death in global and focal ischemia occurs by a cascade of events that unfold temporally and spatially (5). Blocking biochemical processes in the excitotoxic cascade is the rationale behind the various therapeutic strategies for treating stroke and will be the framework for this chapter as we describe therapeutics that have been tested, or are currently being tested, in the clinic.

THERAPEUTIC STRATEGIES FOR TREATING STROKE

Neuronal damage and death do not occur immediately after ischemia, which suggests temporal thresholds for reperfusion as with tPA and possibly therapeutic thresholds for neuroprotection. Thrombolytic therapy attempts to reestablish blood flow in ischemic regions with the aim of preventing or minimizing cell damage (6,7). Various agents have been used in clinical trials to restore cerebral blood flow

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(Table 93.1). All of the agents designed to restore blood flow are inappropriate for treating hemorrhagic stroke because of the danger of exacerbation of cerebral bleeding (8). In fact, most of the drugs that restore blood flow are associated with an increased risk of hemorrhage particularly when administered late (>6 hours). The drugs used to restore blood flow can generally be grouped into antithrombotics, antiplatelet agents, fibrinogen depleting agents, or thrombolytics (Table 93.1).

Antithrombotics such as heparin and warfarin have been tested in numerous trials. There is no evidence to date to support the use of warfarin in the treatment of acute stroke (9). Heparin, followed by coumadin, is an extremely important modality to prevent secondary strokes, particularly cardioembolic stroke, in certain circumstances arterial occlusion following dissection, and nearly always following venous infarction. In a large-scale randomized trial comparing heparin with the antiplatelet aspirin, heparin was associated with only three fewer deaths per 1,000 at 14 days and no difference at 6 months (10). Although there were fewer recurrent strokes within 14 days, more of them were hemorrhagic. Moreover, 12,500 IU heparin was associated with significantly more transfused or fatal extracranial bleeds, hemorrhagic strokes, and deaths than 5,000 IU (10). Low molecular weight heparinoids have been developed that have greater bioavailability and less effect on platelet function than heparin, thus reducing complications such as hemorrhage and thrombocytopenia (11). Results from the trials of two of the low molecular weight heparinoids have been reported. ORG 10172 (Danaparoid) was tested in the Trial of ORG 10172 in Acute Stroke (TOAST) (12). Despite an apparent positive response at 7 days, there was no significant improvement in favorable outcome at 3 months (12). In contrast, Nadroparin (Fraxiparine) was effective in improving outcomes at 6 months when treatment was initiated within 48 hours of symptom onset for a period of 10 days (13). Unfortunately, this study has not been replicated. The results of a 1,500-patient phase III trial (Tinzaparin in Acute Ischemic Stroke Trial [TAIST]) for a third low molecular weight heparinoid, tinzaparin (Innohep), are completed and are currently being analyzed (14).

Antiplatelet agents such as aspirin have also been tested in numerous trials (Table 93.2). Two large-scale phase III trials (Chinese Acute Stroke Trial [CAST] and International Stroke Trial [IST]), in which there were 20,000 patients in each, found that there was a small but worthwhile improvement in the main outcome measures (absolute risk reduction of <1% [need to treat 111 to benefit one]) at 6 months and that aspirin should be started as soon as possible after the onset of symptoms (10,15). In the IST trial, there were fewer deaths and recurrent ischemic strokes with no significant excess of hemorrhagic strokes among the aspirin-allocated patients (10). Recently, the Abciximab in Ischemic Stroke Investigators (16) reported that Abciximab (ReoPro), a potent parenterally administered platelet glycoprotein IIb/

TABLE 93.1. CLINICAL TRIALS FOR ACUTE STROKE TREATMENT[a]

- *Drugs to improve blood flow*
  - Antithrombotic
    - Heparin
    - Nadroparin (low molecular weight heparin)
    - Tinzaparin (low molecular weight heparin)
    - Danaparoid (low molecular weight heparinoid, Org 10172)
  - Anti-platelet
    - Aspirin
    - Abciximab
  - Fibrinogen depleting
    - Ancrod
  - Improve capillary flow
    - Pentoxifylline
  - Thrombolytics
    - Pro-urokinase
    - Tissue plasminogen activator
    - Streptokinase
    - Urokinase
- *Drugs to protect brain tissue (neuroprotective agents)*
  - Calcium channel blockers
    - Nimodipine
    - Flunarizine
  - Free radical scavengers—antioxidants
    - Ebselen
    - Tirilazad
    - NPY-059
  - GABA agonists
    - Clomethiazole
  - Glutamate antagonists
    - AMPA antagonists
      - GYKI 52466
      - NBQX
      - YM90K
      - YM872
      - ZK-200775 (MPQX)
    - Kainate antagonist
      - SYM 2081
    - NMDA antagonists
      - Competitive NMDA antagonists
        - CGS 19755 (Selfotel)
      - NMDA channel blockers
        - Aptiganel (Cerestat)
        - Dextrorphan
        - Dextromethorphan
        - Magnesium
          - Memantine
          - MK-801
          - NPS 1506
          - Remacemide
          - AR-R15896AR
          - HU-211
        - Glycine site antagonists
          - ACEA 1021
          - GV150526
        - Polyamine site antagonists
          - Eliprodil
          - Ifenprodil
      - Growth factors
        - Fibroblast Growth factor (bFGF)
      - Leukocyte adhesion inhibitor
        - Anti-ICAM antibody (Enlimomab)
        - Hu23F2G
      - Nitric oxide inhibitor
        - Lubeluzole
      - Opioid antagonists
        - Naloxone
        - Nalmefene
      - Phosphatidylcholine precursor
        - Citicoline (CDP-choline)
      - Serotonin agonists
        - Bay × 3072
      - Sodium channel blockers
        - Fosphenytoin
        - Lubeluzole
        - 619C89
      - Potassium channel opener
        - BMS-204352

[a]From: *http://www.neuro.wustl.edu/stroke/stroke-drug-categories.htm#ACUTE STROKE THERAPY.*

TABLE 93.2. CLINICAL TRIALS INVOLVING ASPIRIN AS A THERAPEUTIC FOR STROKE[a]

Multicentre Acute Stroke Trial-Italy (MAST-I)
Stroke Prevention in Atrial Fibrillation II (SPAF II)
International Stroke Trial (IST)
Acute Ischemic Stroke Trial—Oral Aspirin versus Intravenous Heparin on Stroke Progression (AIST-ASH)
Chinese Acute Stroke Trial (CAST)
Carotid Artery Stenosis with Asymptomatic Narrowing: Operation Versus Aspirin (CASANOVA)

[a]From: *http://www.neuro.wustl.edu/stroke/therapy/Therapy_1Page5.html*

IIIa antagonist, when administered up to 24 hours after stroke onset, resulted in an improved functional outcome. At 3 months, there was a trend toward a higher rate of minimal residual disability (Barthel Index ≥95 or modified Rankin scale ≤1) among Abciximab patients compared with those who received placebo. Antiplatelets may prove a viable therapy for both prevention and intervention.

Ancrod (Viprinex/Arvin) is a fibrinogenolytic agent that comes from the venom of the Malaysian pit viper (17). Ancrod has recently completed a North American phase III trial (Stroke Treatment with Ancrod Trial [STAT]). Treatment was a 5-day paradigm initiated within 3 hours of onset (18). There was an increase in symptomatic intracranial hemorrhage (5.2% versus 2% for placebo), and there was no difference in mortality at 3 months (18). There is a European trial (ESTAT) for Ancrod that has yet to be completed but BASF, who was jointly conducting the phase III trials, has decided not to continue the trials after an independent group looked at the interim results and failed to see any evidence of efficacy (19).

Thrombolysis has been performed with a variety of compounds administered by various routes. From numerous trials, one thing is clear of all agents in this category: They must be given early or serious hemorrhagic complications are likely (7). Urokinase (uPA) is an endogenous proteolytic enzyme, secreted as a proenzyme, which converts circulating plasminogen to plasmin, producing a systemic lytic state. Intraarterial administration of pro-urokinase (the single chain precursor of urokinase) was evaluated in the Prolyse in Acute Cerebral Thromboembolism Trial (PROACT) with high- or low-dose heparin (20) and in the PROACT II trial with low-dose heparin (21). Although prourokinase and intravenous high-dose heparin resulted in an increased rate of hemorrhage, intraarterial pro-urokinase with low-dose heparin significantly improved the proportion of good outcomes from 25% to 40% and hemorrhage was seen in 10% of patients, consistent with other thrombolytic trials.

Streptokinase is derived from B-hemolytic streptococci and it converts plasminogen to plasmin following the formation of a complex with plasminogen. Metaanalysis of the three major trials of streptokinase, Multicentre Acute Stroke Trial–Europe (MAST-E), the Australian Streptokinase (ASK) trial and the Multicentre Acute Stroke Trial–Italy (MAST-I), showed an increased risk of hemorrhage and death (22,23). The trials were all terminated prematurely.

Intravenous administration of tissue plasminogen activator (tPA) within 3 hours of symptom onset has been approved for the treatment of acute ischemic stroke in the United States for 4 years and it remains the only approved treatment for acute ischemic stroke. There have been four large trials of Alteplase, the European Cooperative Acute Stroke Studies (ECASS I and II), the National Institutes of Neurologic Disorders and Stroke (NINDS) trial, and the Alteplase Thrombolysis for Acute Noninterventional Therapy in Ischaemic Stroke (ATLANTIS). The metaanalysis of these trials shows a significant reduction in death and disability (160/1,000 or a number needed to treat of 6 versus 9/1,000 with a number needed to treat of 111 for aspirin). More trials are needed to gain a better understanding of the timing, safety, and efficacy of Alteplase in different patient populations. However, a recent study has demonstrated the efficacy of tPA for acute stroke in a community setting (24). It remains unclear whether intravenous (IV) or intraarterial (IA) tPA delivery is superior in acute ischemic stroke treatment (7,25–30). IV therapy is faster and more convenient, whereas IA therapy allows for mechanical disruption and higher drug concentration at the clot site (7). A protocol was recently tested in which severe stroke patients with little or no CT changes were given IA tPA following IV tPA if there was no clinical improvement and a persistent occlusion was identified on transcranial Doppler (30). Combined therapy was performed on nine patients and no intracerebral hemorrhages or significant systemic bleeding complications occurred. Marked clinical improvement was noted in four patients, suggesting that combined IA and IV tPA therapy is feasible and safe and that future studies should consider combining rather than comparing these two delivery strategies (30). There is an NIH-approved trial (Interventional Management Study, IMS trial) comparing IV versus combined IV and IA tPA therapy that will be underway by the time this is published.

NEUROPROTECTIVE AGENTS

Once the excitotoxic cascade has begun, the first therapeutic strategy, and the one that has raised the most hope in the last decade, utilizes neuroprotective agents targeting mediators in the excitotoxic cascade (Fig. 93.1). The cascade can be separated spatially and temporally. The cascade begins with an increase in presynaptic calcium influx, followed by glutamate release, postsynaptic glutamate receptor activation resulting in sodium and calcium influx, which results in postsynaptic depolarization and further calcium influx.

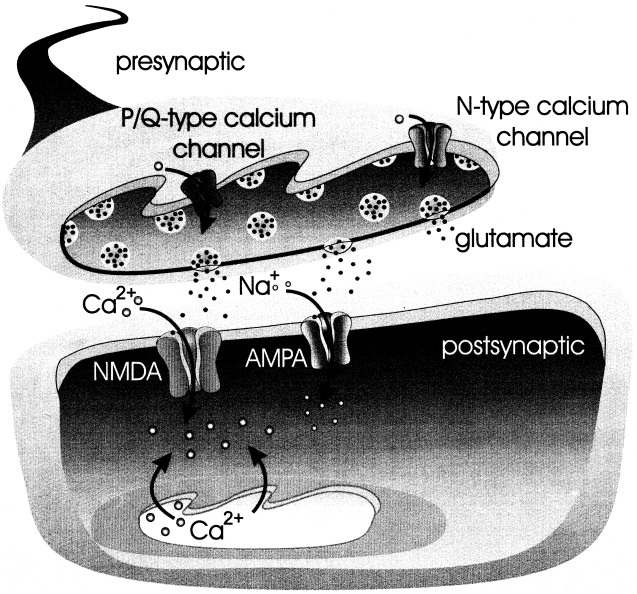

FIGURE 93.1. Thereapy utilizing neuroprotective agents targeting mediators in the excitotoxic cascade.

Voltage-gated sodium and potassium channels are targets that affect depolarization, whereas calcium channels mediate calcium influx and affect depolarization. Most of the neuroprotective agents tested in the clinic have targeted either voltage-gated calcium channels or glutamate receptors, particularly the NMDA receptor subtype. In addition, GABA receptor agonists attenuate excitotoxicity (31) and free radical scavengers are neuroprotectants aimed at the later stages of the excitotoxic cascade (32). After the excitotoxic cascade has progressed, an inflammatory response occurs in which there is infiltration of leukocytes and monocytes (33). Microglia and astrocytic glial cells are activated and macrophages begin responding to chemoattractants. Still other therapeutic strategies have targeted leukocyte adhesion (34,35) and nitric oxide production (36,37). Once much of the damage has occurred and neuroprotection is no longer a viable strategy, neural regeneration and trophism becomes an option. This approach has been mounted with infusion of growth factors but trials to date have been unsuccessful (38). We now look at trials of compounds targeted to these various processes in the excitotoxic cascade.

CALCIUM CHANNEL ANTAGONISTS

Clinically, L-type calcium channel antagonists have been used extensively for the treatment of cardiovascular disorders such as hypertension; however, very few have been tested in the arena of stroke therapy. Although the meta-analysis of oral nimodipine (Nimotop) trials demonstrated no significant effects on neurologic or functional outcome, the drug was administered within a very large therapeutic window (i.e., 24 to 48 hours) following symptom onset (39). More recently, a more aggressive trial (Very Early Nimodipine Use in Stroke, VENUS) designed to treat with nimodipine within 6 hours of symptom onset was terminated before completion because the results indicated that there was no beneficial role of early administration of oral nimodipine at 3 months (40). Although nimodipine failed to demonstrate efficacy in treating acute stroke, it was approved for the treatment of subarachnoid hemorrhage more than a decade ago. The results of a phase III trial of flunarizine (Sibelium) for acute stroke suggested that it also did not improve neurologic or functional outcome at 3 months when administered early (<6 hours) (41).

The N-type calcium channel antagonist, SNX-111 (Ziconotide), preferentially blocks presynaptic calcium channels and inhibits neurotransmitter release. In both focal and global animal models of cerebral ischemia SNX-111 is highly neuroprotective, even when administered after a delay of 24 hours following reperfusion (42). SNX-111 has also been tested in clinical trials for acute stroke. Although the stroke trials of SNX-111 were discontinued because of severe hypotension that exacerbated the ischemic damage (43), SNX-111 has progressed to phase III trials for head trauma (44,45) and has just been approved by the FDA for the treatment of pain (46). Spider toxin antagonists of the P/Q-type neuronal calcium channels are neuroprotective *in vitro* but their *in vivo* toxicity in animals, primarily respiratory depression causing death, has limited their clinical development. However, efforts to generate small peptide analogues of these spider toxins, which exhibit efficacy *in vitro,* are ongoing.

GLUTAMATE RECEPTOR ANTAGONISTS

Numerous clinical trials have been carried out for NMDA receptor antagonists based on preclinical testing in animal models of cerebral ischemia (47). All the phase III trials to date have failed. Optimism for the use of NMDA receptor antagonists in the treatment of acute ischemia has waned and has even prompted some pharmaceutical companies to abandon efforts to develop therapeutics for acute stroke. The experience with NMDA receptor antagonists in the clinic has been that most NMDA receptor antagonists result in psychosis as a common adverse effect (48). NMDA receptor antagonists with greater specificity for various binding sites on the receptor, or selectivity for a given receptor subunit, are being developed which demonstrate greater safety and fewer adverse effects (49–52).

NMDA receptors are heteromeric pentamers composed of at least one NR1 subunit and one or more of the four different NR2 subunits, NR2A, NR2B, NR2C, or NR2D. There are a number of sites on the NMDA receptor at

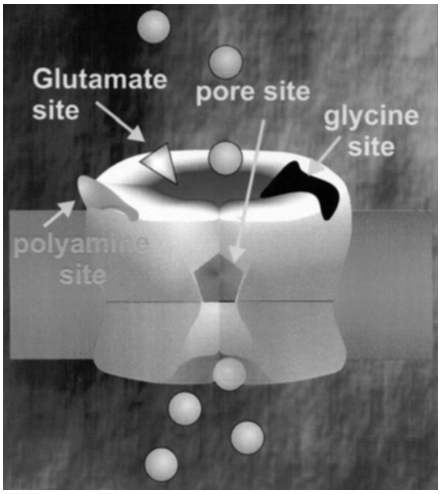

FIGURE 93.2. Sites on the NMDA receptor at which antagonists can bind.

which antagonists can bind (Fig. 93.2). Competitive antagonists bind to the same site as NMDA or glutamate. Glycine and polyamines each bind as activators of the NMDA receptor and there are antagonists of these two sites, respectively. There are also noncompetitive antagonists that bind to the inside of the pore of the channel and sterically inhibit the influx of ions (53–55). These compounds are called use-dependent. Competitive NMDA receptor antagonists block channel activity best when glutamate levels are low. These antagonists would be expected to be less efficacious during ischemia compared to a normally functioning brain because glutamate levels rise during ischemia (56); therefore, the therapeutic index of these agents would be expected to be low.

Not surprisingly, two phase III trials (Acute Stroke Trials Involving Selfotel Treatment [ASSIST] and a head injury trial) of the competitive NMDA receptor antagonist CGS 19755 (Selfotel) were suspended given that they were not effective and caused neurotoxic side effects (57–59). The principal side effects were agitation, hallucinations, and psychotomimetic effects similar to those seen with phencyclidine (PCP) (60,61). Furthermore, among the patients with severe stroke, there was a trend toward a significant difference in the number of deaths between those treated with Selfotel (33%) and those in the placebo group (22%) (58).

Two years later, phase III trials for the noncompetitive NMDA receptor antagonist Aptiganel (Cerestat/CNS-1102) were discontinued (62). In a subsequent analysis of the phase III trial, some potential therapeutic benefit was identified in a subset of the stroke population. Cambridge Neuroscience plans to further investigate these beneficial effects in partnership with Boehringer Ingelheim (63). The most common side effects with this high-affinity channel blocker were motor retardation, elevated blood pressure, perceptual disturbances, and hallucinations, similar to those seen with Selfotel (60). The rest of the noncompetitive NMDA receptor antagonists exhibit much lower affinity for the ion channel pore than Cerestat. Although memantine has been shown to be neuroprotective in both *in vitro* and *in vivo* models (64) and memantine is progressing for the treatment of dementia (65), it is not currently being developed for the treatment of acute stroke to our knowledge. Remacemide and its active desglycinyl metabolite are well-tolerated at relatively high doses in humans, have demonstrated significant neuroprotective efficacy in animal models of cerebral ischemia, and were doing well in phase II trials for acute stroke (66); however, there is some question as to whether optimal neuroprotective doses would be achieved within the early hours of treatment in humans (51). Thus, the low-affinity compound ARR-15896 was developed as a backup to remacemide and is currently in early Phase II trials (66). Hu-211 (dexanabinol, Cypros) is a nonpsychogenic cannabinoid with both low affinity use dependent block of NMDA receptors, as well as inhibition of tumor necrosis factor-α and antioxidant properties. It exhibits widespread neuroprotective actions in several animal models of stroke and head trauma (67) and has just recently completed a small phase II trial for head trauma clearing the way for a large multinational clinical trial of several hundred severe head trauma patients to commence later this year (68). Magnesium, also a low-affinity noncompetitive NMDA receptor antagonist, is currently in phase III clinical trials for the treatment of acute stroke (Intravenous Magnesium Efficacy in Stroke [IMAGES]). Patients are randomized to receive placebo or intravenous magnesium (16 mmol over 15 minutes followed by 65 mmol over 24 hours) within 12 hours of symptom onset (69). Recruitment of the 2,700 patients is ongoing and should be completed by 2002. Magnesium infusions are well tolerated in humans and have been demonstrated to be neuroprotective in animal models of cerebral ischemia (49,69,70). In a smaller trial, magnesium-treated patients improved neurologically and the need for institutional care 6 months after the stroke was reduced (49,71).

NPS-1506 is another open channel blocker being developed for the treatment of acute stroke. NPS-1506 acts at a novel site within the pore and is an orally active small molecule developed by drawing on the knowledge of spider toxins with pore-blocking abilities (72). NPS-1506 is currently in phase Ib and to date is devoid of the side effects normally attributed to noncompetitive antagonists. In animal models of cerebral ischemia, significant protection is achieved even when NPS-1506 is administered up to 2 hours after onset of stroke. At neuroprotective doses there were no PCP-like behavioral effects, no learning or memory impairment, no neuronal vacuolization, and no significant sedation or cardiovascular side effects (72,73).

Polyamine site NMDA receptor antagonists like eliprodil (SL 82-0715) and ifenprodil have also not done well in

clinical trials (54). Their failure has been attributed to the cardiovascular side effects they possess by virtue of their affinity for sodium and calcium channels in addition to NMDA receptors (74,75). Eliprodil had electrocardiographic effects that limited dosing such that efficacy was not obtained and phase III trials were abandoned in 1997 (74). Another feature of the polyamine site antagonists is that they are relatively specific for NR2B receptor subunits (76). The affinity of eliprodil for NR2B subunits is more than 100-fold greater than that for NR2A or NR2C receptor subunits. A number of other NR2B subunit selective antagonists are currently under development for the treatment of stroke (Table 93.3). Ro 25-6981 and CP-101,606 are both high-affinity NR2B-selective antagonists with very slow kinetics (76–78). CP-101,606 is neuroprotective in animal models of cerebral ischemia but the slow kinetics may limit the rate at which neuroprotective doses may be achieved in human stroke. The fact that there were no psychotomimetic effects of CP-101,606 in phase II trials for moderate head injury and hemorrhagic stroke and that the drug was well tolerated suggest that CP-101,606 might be well tolerated in occlusive stroke (79). Compounds such as Co 101244/PD 174494 are being developed with high-affinity antagonists such as CP-101,606 but more rapid kinetics like isoxsuprine in anticipation of the slower kinetics of CP-101, 606 presenting problems in efficacy in treating stroke (80). Something else which may increase the chances of success of Co-101244/PD174494 is that it also had much less affinity for α1-adrenergic receptors and displayed a reduction in inhibition of potassium channels, something that most other high-affinity compounds possess (80).

Glycine site antagonists exhibit better safety profiles than NMDA receptor antagonists that bind to other sites (48). Most of the clinical attention on glycine site antagonists has been focused on two therapeutic candidates, ACEA 1021 (Licostinel) and GV150526. ACEA 1021 (5-nitro-6,7-dichloro-2,3-quinoxalinedione) is a member of a family of compounds called kappagems. ACEA-1021 has demonstrated neuroprotective efficacy in animal models of focal and global ischemia (81) and it exhibits minimal adverse CNS or cardiovascular side effects (50,82). The compound, originated by ACEA, was being developed by CoCensys for Novartis, but Novartis stopped participating in its development after crystals of ACEA-1021 were found in the urine of some participants in a phase I study (83). CoCensys retains the rights to the drug and is continuing to evaluate it in phase II trials. One approach to dealing with the problems of ACEA-1021 excretion has been to treat in combination with probenecid, which nonselectively inhibits secretion of anionic compounds (84). The combination of ACEA-1021 and probenecid resulted in significantly larger infarct reductions in animal models of cerebral ischemia suggesting that the limiting factor in ACEA-1021 efficacy is related to the steady-state levels that can be elevated by combination therapy with probenecid (84).

GV150526 is significantly neuroprotective in animal models of cerebral ischemia and like ACEA-1021, has shown good tolerability with minimal CNS side effects in the Glycine Antagonist in Neuroprotection (GAIN) phase I and II trials (The GAIN Investigators) (52). Minor abnormalities in liver function were noted with higher maintenance doses, but these changes were asymptomatic and re-

TABLE 93.3. NR2B SUBUNIT-SPECIFIC NMDA RECEPTOR ANTAGONISTS[a]

Subunit Compound	NR2A	NR2B	NR2C	Cortical Neurons (Mostly NR2B)	Rank Order Kinetics
Isoxsuprine (Sigma I-0880)	—	0.7	—	—	Fast
Nylidrin[b]	27	0.19	33	0.2	Medium
Ifenprodil (Synthelabo)[b]	84	0.31	130	0.12	Medium
	40[a]	0.25[a]			
Eliprodil (Synthelabo)	>100	1	>100	0.6	Fast
	50[a]	0.7[a]			
Co-101244/PD174494		0.02	—	—	Medium
Haloperidol	>300	3.1	>300	1.5	?
Ro 25-6981 (Roche)[b]	60[a]	0.009[a]	—	—	Slow
CP 101,606 (Pfizer)[b]	100[a]	0.1	—	—	Slow
		0.06[a]			
Glutamate	4[a]	1.3[a]	—	—	—

[a]All numbers are IC50s in μM and are from Whittemore et al., 1996 unless indicated by[a], in which case they are from Trube et al., 1996. The more potent, the slower the kinetics but also the more protective. From Whittemore ER, Ilyin VT, Woodward RM, Subtype-selective antagonism of NIMDA receptors by Nylidrin. *Soc Neurosci Abat* 1996;22:506–507 and Trube G, Elrhard P, Huber G. The selectivity of RO 25-6981 for NMDA receptor subtypes expressed in Xenopus oocytes. *Soc Neurosci Abat* 1996;22:691–693.

[b]Glutamate dependence: lower affinity with lower glutamate, than with higher glutamate concentrations. All IC50s are done with high glutamate.

solved within 10 days (52). The results of the dose-escalation phase II clinical trial for GV150526 (85) were reported last year and were followed with the GAIN Americas and the GAIN International phase III trials. Approximately 1,600 patients were recruited into randomized double-blinded placebo controlled trials. Two substudies in each trial were planned, to measure lesion volume by MRI-DWI (Magnetic Resonance Imaging-Diffusion Weighted Imaging) and to measure health-related quality of life outcomes. The results of the GAIN International were presented at the 25th International American Heart Association meeting and were less than anticipated and completely neutral. Unlike most trials with NMDA receptor antagonists, the problem with GV150526 was not safety but rather efficacy (85). The results from the GAIN American study are not yet available.

In contrast to NMDA receptor antagonists, there is little experience using AMPA and KA receptor antagonists to treat acute stroke. Fewer binding sites have been characterized on the AMPA receptor compared to the NMDA receptor. Competitive quinoxalinedione antagonists bind to the AMPA binding site and there are also noncompetitive GYKI [GYKI 52466,1-(4-aminophenyl)-4-methyl-7,8-methylenedioxy-5H-2,3-benzodiazepine HCl] site-binding antagonists, benzodiazepine binding-site antagonists, and pore-blocking antagonists (86). The pore-blocking antagonists are mostly from spider toxins and are not appropriate for clinical development. There is one noncompetitive AMPA/KA receptor antagonist, EGIS-9637, which demonstrates neuroprotective efficacy in both focal and global animal ischemia models, but the clinical development has not begun and little is known of the tolerability (87). One of the more common CNS effects of AMPA receptor antagonists is sedation. All of the AMPA receptor antagonists in clinical trials for the treatment of acute stroke are competitive antagonists based on the quinoxalinediones such as NBQX. NBQX and other quinoxalinediones are neuroprotective in animal models of ischemia, even when administered up to 12 hours following reperfusion (88). The main problem with quinoxalinediones has been nephrotoxicity owing to the poor solubility of these compounds (89). One AMPA receptor antagonist, ZK-200775, progressed to phase IIa but these trials were discontinued in 1998 owing to excessive sedation (90). Yamanouchi Pharmaceuticals have developed a series of competitive AMPA receptor antagonists that are neuroprotective in animal models and exhibit improved solubility (91–93). In phase I trials YM90K was well tolerated and in spite of a high rate of urinary excretion there were only mild changes in kidney function markers with a single intravenous dose of 36 mg and repeated doses of 24 mg over 3 hours (94). These phase I trials were followed by phase II trials using the AMPA receptor antagonist, YM872, which has a solubility 800-fold greater than NBQX or YM90K at pH 7 (95). Phase IIa trials are ongoing. The only adverse effect reported in the YM872 phase I study was euphoria in some patients at the higher dose levels (96). The challenge with YM872 will be overcoming the short half-life of the drug to maintain the elevated plasma levels necessary to effect neuroprotection.

GABA RECEPTOR AGONISTS

Clomethiazole (Zendra), a GABA receptor agonist, has just completed large-scale phase III clinical trials for the treatment of acute stroke, Clomethiazole Acute Stroke Study (CLASS and CLASS-I, H and T) (97). Clomethiazole was well tolerated and appeared safe. Sedation was the most common adverse event that led to treatment withdrawal in almost 16% of treated, compared to only 4% in the placebo group. In the main efficacy analysis 56.1% of patients taking clomethiazole and 54.8% of placebo patients reached relative functional independence (97). The difference was not significantly different but in a subgroup analysis of 545 patients who had total anterior circulation syndrome (TACS), the percentage of those reaching relative functional independence was 40.8% on clomethiazole and 29.8% in the placebo group (98). This subgroup efficacy has prompted the testing of clomethiazole in large ischemic cerebral infarctions (CLASS-I), ischemic infarctions in those who receive tPA (CLASS-T), and intracerebral hemorrhage (CLASS-H). Previous failed clinical trials for stroke may have shown efficacy had there been subgroup targeting like that being carried out in the CLASS-I study. However, it is difficult to categorize subgroups of strokes while operating under the extreme time constraints stroke treatment requires. A recent protocol for grading strokes has been developed (ASPECTS, which addresses the need for rapid diagnosis and assessment of infarct (99).

OTHER THERAPEUTIC TARGETS

Voltage-Gated Channels

Potassium channel openers may rescue injured penumbral neurons, thus reducing the size of the infarct and improving neurologic outcome. The novel potassium channel opener, BMS-204352, is being assessed in the Potassium-channel Opening Stroke Trials (POST) in which medication is administered within 6 hours of onset of symptoms. The primary endpoint is change in lesion volume at 12 weeks determined by diffusion/perfusion MRI.

Opioid Receptor Antagonists

The competitive κ-selective opioid receptor antagonist Cervene (Nalmefene) is neuroprotective in animal models of

ischemia and clinically safe (100). In a phase II trial in patients with acute ischemic stroke treated with 6, 20, or 60 mg Cervene there was no significant functional improvement compared to placebo after 3 months (100). A secondary analysis showed an improved 3-month outcome in patients less than 70 years of age.

Free Radicals, Nitric Oxide, and Inflammation

During reperfusion, oxygen-free radicals contribute to damage neurons when antioxidant defense mechanisms are not optimal. Superoxide, hydrogen peroxide, and hydroxyl radicals are responsible for damaging membranes and degrading DNA. In experimental models, agents such as vitamin E, glutathione, superoxide dismutase, iron chelators, lazaroids, NPY-059, and catalase have been tested as free radical scavengers. One of the most tested agents, Triliazad mesylate (Freedox), an inhibitor of lipid peroxidation, is neuroprotective in stroke models (37). In a phase II study of over 400 patients treated with 6 mg/kg per day tirilazad for 3 days within 6 hours of onset, no significant outcome relative to placebo control was observed (101). The Randomized Trial of High Dose Tirilazad in Acute Stroke (RANTTAS II) phase III trials of a higher dose was stopped because of a lack of efficacy (102). Similarly, the antioxidant Ebselen, which has glutathione peroxidase-like activity, showed no efficacy (103). NPY-059 is currently in early phase II trials by Centaur.

Lubeluzole (Prosynap) is a benzothiazole that blocks glutamate-induced nitric oxide-mediated neurotoxicity in rats. In a phase II trial patients presenting within 6 hours were randomized to a placebo group or to receive iv Lubeluzole at 7.5 or 15 mg over 1 hour as a loading dose, followed by a continuous infusion of 10 or 20 mg per day for 5 days, respectively. This trial was terminated early owing to high mortality in the high-dose group (36). Clinical development was subsequently stopped after a large phase III trial (LubInt-13) failed to show efficacy.

Citicoline provides cytidine and choline as precursors in the synthesis of the integral membrane component phosphatidylcholine. During ischemia phosphatidylcholine is broken down into free fatty acids, which in turn generate free radicals. Citicoline has been studied as a neuroprotectant as well as a promoter of neural plasticity and repair after stroke through its actions to promote membrane synthesis, to decrease the levels of free fatty acids and to promote the regrowth of axons and nerve terminals. In a phase II study, oral citicoline (CerAxon; 500, 1,000, and 2,000 mg per day) was administered within 24 hours for a 6-week period (104). A dramatic improvement in functional independence outcome (61% versus 39% in the placebo group) was seen in the 500 mg and 2,000 mg (but not the 1,000 mg) dose groups. A second study of 500 mg per day citicoline versus placebo failed to demonstrate significant differences in outcome or mortality. The results from the 899 patient phase III trial comparing 2,000 mg citicoline to placebo (105) failed to meet the primary endpoint, an improvement in neurologic function. However, citicoline was shown to be safe and there was a higher percentage of citicoline-treated patients with reduced infarct volumes compared to the placebo group.

Treatment with a monoclonal anti-ICAM-1 antibody inhibits leukocyte adhesion by blocking leukocyte attachment and migration through cerebral endothelial cells and reduces neurologic deficits in rodent ischemia models (34). The Enlimomab Acute Stroke Trial (EAST) completed in 1996 showed that the anti–ICAM-1 antibody treated patients had a worse outcome than placebo-treated patients (35). LeukArrest (Hu23F2G) is another human antibody that inhibits neutrophil movement from the blood into the brain by specifically targeting CD11/CD18 molecules on the surface of neutrophils. A recently completed phase II trial showed LeukArrest to be safe and suggested an improved neurologic outcome. The Hu23F2G Phase III Stroke Trial (HALT) with 310 patients was begun in early 1999.

CONCLUSION AND FUTURE DIRECTIONS: THE ARSENAL OF A UTOPIAN STROKE CARE CENTER

Given the worldwide efforts focusing on cloning the human genome and the parallel efforts to identify disease specific genes and proteins through genomics and proteomics, we have no way of knowing how many stroke specific genes and proteins will be identified. Estimates of the total number of disease genes are between 10,000 and 30,000. Because there are currently only 450 clinical targets of therapeutic intervention worldwide, there are at least 9,000 new genes for which we have no idea of function and no pharmacologic tools with which to study them. One thing is certain; we will be faced with an explosion of information in 2001, the expected completion date of the human genome project.

In terms of the strategies described in the preceding, efforts to increase blood flow may include angiogenesis using a recombinant adenovirus expressing vascular endothelial growth factor (VEGF) or perhaps other gene therapy delivered vascularly. Still more effort is being focused on NMDA receptors, but in developing safer compounds such as ARL 15896 and GV150526 with a better therapeutic ratio than agents that previously failed in trials. Trials are well underway for some AMPA receptor antagonists like YM872 and the results of these will reveal whether this strategy is a viable one worthy of further attention.

Growth factor therapies are targeted to improve neuronal survival, repair, and plasticity following acute ischemic stroke. A phase III trial of fibroblast growth factor (Biblast, bFGF, Trafermin) was discontinued because of no efficacy

relative to the placebo group. The glial derived growth factor, GFG2, is being developed by Cambridge NeuroScience (CNSI) for treating neurodegenerative disorders and may perhaps treat stroke (106). Another avenue of therapy is to promote the growth of new vessels through angiogenesis, but this too has yet to be realized in trials for stroke. Neurogenesis is an area that is generating attention in animal models but the growth of new neurons occurs very late and experimentally only in the dentate; therefore, the relevance to the clinical situation is somewhat obscure (107). A viable alternative to promoting the growth of the brain's own cells is to treat with stem cells that have the potential of generating new neurons. Recently this was successfully attempted *in vivo* (108).

Although the recent Heart Outcomes Prevention Evaluation (HOPE) trial, which looked at ramipril versus vitamin E, failed to demonstrate effects of vitamin E on any of the cardiovascular outcome measures, including myocardial infarction, unstable angina, congestive heart failure, stroke, and death from cardiovascular causes (109), two new trials, Vitamin Intervention for Stroke Prevention or VISP (110) and Vitamins to Prevent Stroke or VITATOPS (111) have recently begun to determine whether daily B_{12}, B_6, and folate, which reduce homocysteine levels, are better than the best medical practice and surgical management alone to reduce the recurrence of vascular events in stroke patients.

The Women's Estrogen for Stroke Trial (WEST) will assess whether postmenopausal estrogen therapy (1 mg estradiol per day) in women with recent transient ischemic attacks or nondisabling stroke can reduce the risk of death or recurrent stroke (112).

Inasmuch as we have laid out spatially distinct strategies, there is an overlapping temporal association of these strategies. This temporal aspect can be exploited in the form of co-therapies. Because ischemic death occurs via a cascade involving several processes, it is likely that targeting one single process will prove inadequate and instead, targeting several of the processes with a cocktail of therapeutic agents given in a specific temporal sequence will prove efficacious. The development of safe therapeutics for the intervention of stroke should provide a brighter future for stroke survivors with the increase in stroke awareness and the number of centers with dedicated stroke care units.

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