MEETING REPORT

Highlights of the SMR Symposium on Stroke, held July 9, 1998, in Windlesham, U.K.

Stroke: Therapeutic Approaches

by Jeremy Gilmore and Roger Horton

Summary

Speakers at the Society for Medicines Research Symposium on Stroke, held July 9, 1998, in Windlesham, U.K., covered topics ranging from reasons for failure of drugs in late-stage clinical trials to *in vitro* and *in vivo* models of ischemia to therapeutic approaches to this disorder. The take-home message was that the side effect profile in humans needs to be closely examined, with emphasis on the pharmacokinetics and distribution to minimize the cardiovascular effects.

On July 9, 1998, the Society for Medicines Research held a one-day Symposium on Stroke, hosted by Eli Lilly and Co. Ltd. at its research center in Windlesham, Surrey, U.K. The treatment of stroke remains one of the great unmet medical challenges, with the long list of failed clinical trials being a reflection on the complexity of the problem. The meeting brought together an international lineup of speakers to discuss current and future approaches to the disorder.

Overview of stroke

Keith Muir (Southern General Hospital, Glasgow, U.K.) provided an overview of the disorder and of recent experiences with clinical trials of new drug candidates. Stroke is the third leading cause of death in the Western world and the primary cause of disability, with no current therapies able to preserve neurons during an ischemic attack. Magnetic resonance imaging (MRI) scanning has demonstrated that in humans the subsequent neurodegeneration in the hypoperfused but still metabolically alive penumbra is a gradual process occurring over a number of days. Clinical trials have repeatedly produced negative outcomes. Thus, agents affecting the thrombotic process, such as **streptokinase**, **heparin** or **aspirin**, have produced negative results due to the increased incidence of cerebral hemorrhage. However, improved outcomes were seen with **recombinant plasminogen activator** (r-tPA), which has now been licensed in the United States. Other notable trial failures include **selfotel** (an NMDA antagonist); **eliprodil** (a polyamine site blocker of the NMDA receptor); **aptiganel** (*Cerestat*; an NMDA antagonist); and **lubeluzole** (a mixed sodium channel blocker/NOS inhibitor) (Fig. 1).

Muir continued by reviewing the reasons why so few drugs have worked in humans and why they have often failed late in clinical trials. During drug development, insufficient note has been taken of the side effect profile. Also, the design of the trials themselves has often been at fault, with no knowledge of the ideal dosing regimen and with recruitment including patients with lacunar strokes who would usually have a benign prognosis (thus accounting for the high placebo rate of around 30%). In phase II trials, only cortical stroke victims should be included in the study design as they account for 20–40% of patients amenable to neuroprotection. A long follow-up period is required to show a significant beneficial outcome. Pretrial screening measures, such as SPECT and diffusion-weighted MRI to identi-

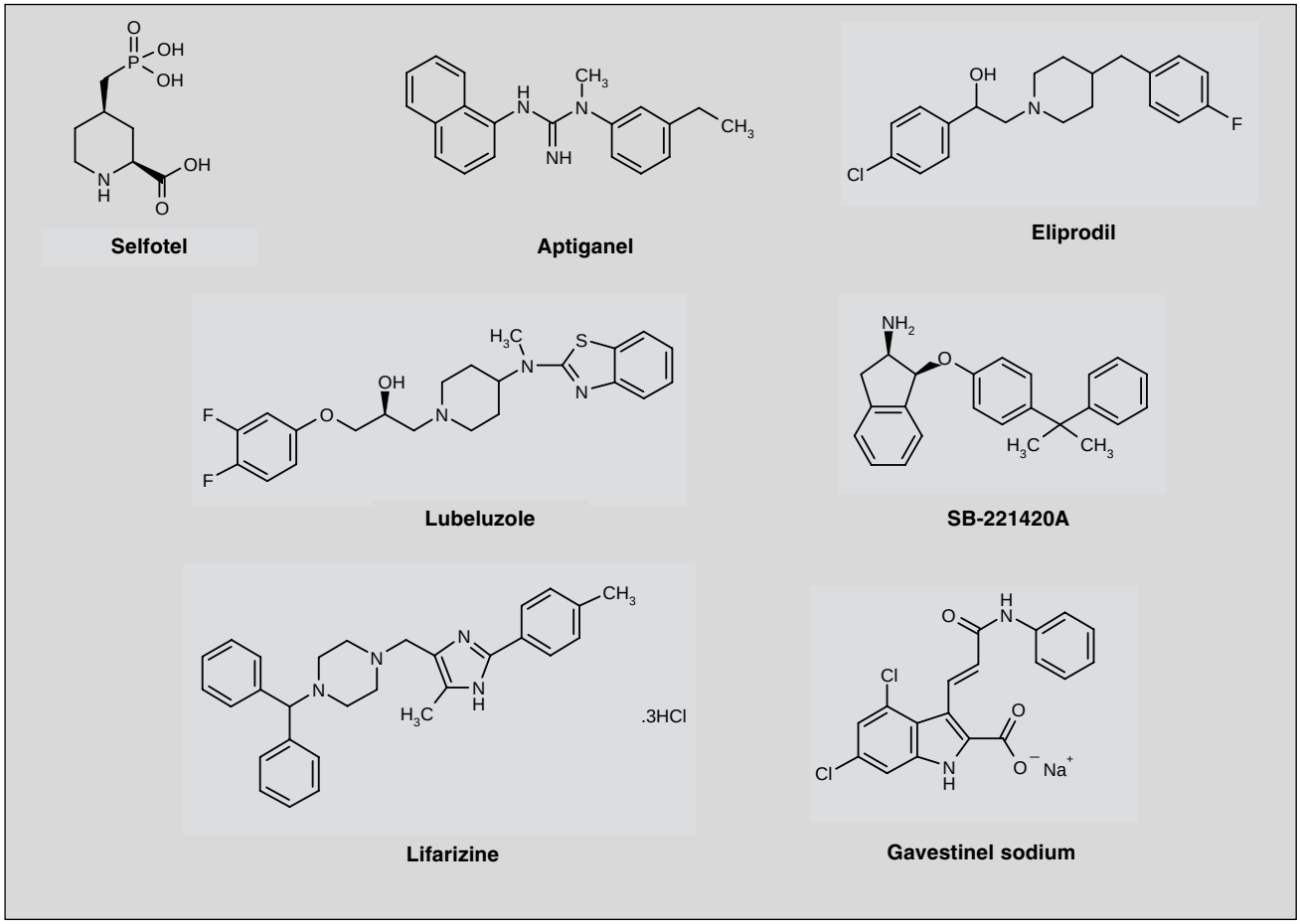

Fig. 1. Structures of some of the compounds discussed at the symposium.

fy residual reperfusion and PET to investigate cerebral blood flow, would aid in the identification of the type of stroke victim and thus the likely outcomes expected in the absence of therapeutic intervention.

Models of ischemia

Jackie Hunter (SmithKline Beecham, Harlow, U.K.) discussed the relative merits of the various *in vitro* and *in vivo* models of ischemia. *In vitro* models have proved of limited usefulness, with the correlation to the *in vivo* situation being poor; however, organotypic hippocampal slice cultures, derived from neonatal tissue, under conditions of hypoxia and/or hypoglycemia, are gaining in popularity as prescreen models prior to *in vivo* assessment.

Hunter then reviewed the various *in vivo* models available. The two main global models (equivalent to a cardiac arrest) are the gerbil bilateral carotid artery occlusion model (BCAO) and the four-vessel occlusion model (4-VO). The focal models (equivalent to an acute stroke) include transient and permanent middle cerebral artery occlusion (MCAO) and thromboembolic models. In the mouse MCAO model, it is important to compare the middle cerebral artery branching patterns, as there are significant differences between the various strains of CD1 and Balb C mice, resulting in poor reproducibility in infarct volume following electrocoagulative occlusion. The permanent MCAO model is seen as the gold standard, while the transient model is easier to reverse but gives increased variability. In addition to looking at the infarct size by histopathology, it is also important to look for behavioral changes (e.g., grid walking activity, forelimb placement errors).

Therapeutic window

An important consideration when looking at a potential new agent is the therapeutic window—is it species dependent? The window for intervention is potentially longer in gyroencephalic species. It may also depend on the mechanism of therapeutic intervention and be affected by reperfusion. The endpoints assessed may not only be histological, but could also use structural and functional imaging, behavioral outcomes (motor, cognitive) and changes in expression patterns of targets of relevance. Hunter reiterated many of Muir's concerns about the lack of correlation between animal models and subsequent human studies, with the conclusion that many clinical failures could have been predicted from proper animal experiments, where it was ensured that drug characterization was undertaken across a number of models and species.

Hunter used results with the nonselective neuronal calcium antagonist **SB-221420A** (Fig. 1) to illustrate a number of the animal models. The compound was stopped in development because of liver toxicity.

Therapeutic approaches

Michael Spedding (Servier, Paris, France) discussed the potential of sodium and calcium channel modulators in the treatment of stroke. In addition to the recognized incidences of stroke, there may be additional problems with silent strokes that are thought to be associated with subsequent dementias. Calcium channels are classified into five types: 1) the L-type blocked by dihydropyridines; 2) the T-type; 3) the N-type blocked by conotoxin MVIIA (**SNX-111**); 4) the P-type blocked by agatoxin IVA; and 5) the Q-type blocked by conotoxin MVIIC (**SNX-230**).

Dihydropyridines such as nimodipine may be useful in hemorrhagic stroke but are precluded from use in ischemic stroke owing to their hypotensive actions. They are also of limited benefit owing to the need to block other channels—Spedding advocated the use of dual sodium and calcium blockade. He exemplified this with a discussion of the actions of **lifarizine** (Fig. 1), a mixed sodium/calcium channel blocker, which was effective in a number of animal models and shown to increase NGF synthesis from astrocytes. Its use was limited by the side effects of prolonged QT_c and hypotension. In a small trial (147 patients) it only produced a marginal effect.

The take-home message was that the side effect profile in humans needs to be closely examined, with emphasis on the pharmacokinetics and distribution to minimize the cardiovascular effects. Spedding proposed that stress could exacerbate the effects of an ischemic insult, a result that has been noted in animal models. Servier has a promising AMPA kainate blocker (**S-17625**) that gives significant reductions in infarct size in animal models without producing respiratory depression and behavioral effects. Its progress is being hampered by kidney toxicity.

Richard Miller (University of Chicago, Chicago, Illinois, U.S.A.) gave an overview of the mechanisms involved in excitotoxicity and apoptosis, illustrating his talk with examples of single-cell imaging studies performed in his own lab. Overstimulation of glutamate receptors leads to excessive calcium influx into neurons, which in turn causes either necrotic or apoptotic cell death. Much stroke-associated neurodegeneration is of the latter type, initiated by a dysfunction of the mitochondria. Following the glutamate-mediated Ca^{2+} uptake, many of these ions enter the mitochondria resulting, among other effects, in the synthesis of excessive concentrations of reactive oxygen species. In addition, the high Ca^{2+} concentrations lead to the opening of a high-conductance ion channel in the inner mitochondrial membrane, called the mitochondrial permeability transition pore (MTP). Opening of the channel discharges the excess Ca^{2+}, but also causes collapse of the mitochondrial membrane and release of cytochrome C, which in turn triggers a cascade of events leading to caspase activation and apoptosis. The immunosuppressive agent **ciclosporin** (or the related nonimmunosuppressant **PKF 211-811**) blocks the MPT and can be shown in an *in vitro* model to have neuroprotective effects. The susceptibility of the mitochondrion to depolarize is modified by a number of gene products, such as Bcl-2, preserving the membrane potential in the face of high [Ca^{2+}] or reactive oxygen species. Modulation of growth factors like TGF-β_1, which increase levels of Bcl-2, has the potential to provide new strategies to stroke therapy.

Romano Di Fabio (Glaxo Wellcome, Verona, Italy) described the identification of **GV-150526A** (**gavestinel sodium**; Fig. 1) as a potent neuroprotective agent. Based on the excitotoxicity hypothesis, overstimulation of NMDA glutamate receptors results in a massive influx of Ca^{2+} into the postsynaptic neuron, leading ultimately to cell death through the activation of neurotoxic cascades. Therefore NMDA antagonists have been proposed as potential therapeutic agents in the treatment of stroke.

Glaxo Wellcome chose to look at glycine site modulators with the expectation of a better side effect profile. Di Fabio described the SAR development from a simple 4,6-dichloroindole-2-carboxylic acid lead structure. In particular, introduction of an acrylamide function at the 3-position of the indole gave selective nanomolar affinity at the glycine binding site. Greatest potency was observed with the phenylamide derivative **GV-150526A** (3 nM binding affinity on rat cortex).

Further QSAR studies, investigating aromatic substitution on the phenyl ring, revealed that activity was related to increasing electron-donating effects and decreasing total bulk and lipophilicity. GV-150526A showed very similar activity to MK-801 in the rat MCAO model when dosed five minutes preocclusion (3 mg/kg i.v.), but importantly showed activity even when dosed six hours postocclusion (ED_{50} = 3 mg/kg). The compound gave no gross behavioral changes, had no effect on memory and in the rotarod test, and gave no neurovacuolization.

Michael Chopp (Henry Ford Hospital, Detroit, Michigan, U.S.A.) presented the importance of inflammatory processes resulting from a focal cerebral ischemic event. Ischemic tissue, through the intermediacy of an orchestrated sequence of adhesion molecules, signals circulating inflammatory cells to invade the site of injury. In the MCAO models, neutrophils are identified in large numbers, with the maximum cell count at 48 hours following transient occlusion and 72 hours following permanent occlusion. An anti-ICAM antibody (**1A29**) gives neuroprotection following a two-hour occlusion when infused for 18 hours. When sacrificed at 24 hours, the animals showed increased levels of E- and P-selectin in vessels within the

ischemic lesion. **rNIF** did not alter infarct volume in the permanent MCAO model, but significantly decreased infarct volume after transient ischemia, suggesting that reperfusion needs to occur.

Chopp presented evidence to support the view that antiadhesion approaches are more effective when combined with thrombolytic agents. In an embolic ischemia model, a **combination of r-tPA and an anti-CD18 antibody** significantly decreased infarct volume at two hours. At four hours the combination remained effective compared to the r-tPA monotherapy, where no protection was seen.

Astrocytes may also play a significant role after an ischemic event. They encircle nonperfused blood vessels. This is associated with a large increase in vascular endothelial growth factor, which may contribute to the angiogenic response. Chopp also discussed the potential of targeting proteins that become elevated after a stroke. He compared the brain after a stroke to that of an infant, in that proteins such as nestin, which are expressed in infancy, are replaced by GFAP during development, but are induced again after ischemia. Apoptosis is normally involved in the developmental stage, and **staurosporine**, a PKC inhibitor, is capable of reducing infarct size and increasing markers of synaptogenesis. Chopp argued that increased emphasis on promoting the brain's ability to repair itself may prove to be a more effective and a more practical strategy for the treatment of stroke than early intervention to prevent neuronal death.

Seth Finklestein (Massachusetts General Hospital, Boston, Massachusetts, U.S.A.) completed the day's talks with a discussion of the role of growth factors in enhancing neuronal plasticity and thus improving functional recovery following a stroke. He initially focused on **fibroblast growth factor** (bFGF) (*Fiblast*®), a 154-amino-acid protein that interacts with a tyrosine kinase receptor and induces axonal growth. It reduces infarct size in the suture occlusive model when given by i.v. infusion (50 μg/kg/hour) 30 minutes postocclusion with a time window of three hours. It is effective in mice, rats and cats. The blood–brain barrier is disrupted after a stroke, and 0.01% of the administered bFGF passes across the barrier. It is necessary to give high doses and therefore cardiovascular side effects and nephrotoxicity are seen. Owing to the latter side effect, a U.S. phase III trial has been halted, but in a European trial where the drug was administered at a lower dose over a longer period, such toxicity issues have been reduced. When administered i.c.v. 24 hours after an experimental stroke, there was no alteration in infarct size, but there was significant functional improvement.

Like Muir, Finklestein commented that only 20–40% of patients have cortical strokes that would benefit from neuroprotection. The remainder of patients have either lacunar strokes or small partial strokes that show complete recovery, or subcortical strokes that are not amenable to drug therapy. Although impractical, the use of imaging techniques (e.g., diffusion-weighted MRI) would confirm cortical infarcts. Failing this, he suggested the use of the Oxfordshire scale to confirm cortical strokes—although slowing patient recruitment, it would increase the power of the study to detect a benefit.

Finklestein also discussed the potential of the growth factor **osteogenic protein-1** (OP-1, a member of the TGF-β family), which induces dendrite growth in the brain without affecting axonal growth. Like bFGF, OP-1 when given intracisternally improved behavioral responses without altering infarct size. Effects were seen even after a single dose given three days postocclusion. Finklestein concluded that enhancing the recovery process following stroke, involving a rewiring of the brain in the contralateral hemisphere, was likely to become an increasingly important strategy.

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IMMUNE RESPONSE PRESENTS PHASE II DATA ON PSORIASIS TREATMENT

The Immune Response Corp. announced September 9, 1998, that it presented the results of an exploratory, double-blind, placebo-controlled phase II trial involving 84 patients with moderate to severe psoriasis at the 5th European Congress on Psoriasis/7th International Psoriasis Symposium on September 5, 1998, in Milan, Italy.

This nine-arm clinical trial was designed to compare the company's potential T cell receptor (TCR) peptide therapeutic vaccine (**IR-502**) using different adjuvants and two different routes of administration in an effort to select the best formulation for future clinical development.

The company believes the results suggest that the groups receiving intramuscular injections of TCR peptides along with incomplete Freund's adjuvant (IFA) showed improvement when compared to all other treatment groups. These other treatment groups included patients receiving i.m. injections of IFA alone, i.m. injections of the TCR peptides in *DeTox*™ adjuvant, intradermal injections of peptides in saline or intradermal injections of saline alone.