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The Continued Promise of Neuroprotection for Acute Stroke Treatment

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Abstract

Stroke is the second leading cause of death. However, effective pharmocologic treatment options are still extremely limited and applicable to only a small fraction of patents. The translational failure in finding an effective neuroprotectant for ischemic strokes has generated an active discussion in this field. One focus has been on validating systems for testing neuroprotectants. This review discusses some fundamental issues in experimental stroke that are worthy of further exploration. We begin with a general review of the current status of experimental stroke research and then move on to a discussion of the determining factors and processes that control and differentiate the fate of ischemic ischemic cells and tissue. We propose several strategies of neuroprotection for ischemic strokes with an emphasis on manipulating cellular energy state.

Keywords: Stroke; Cerebral ischemia; Neuroprotection; Penumbra; Treatment; Energy state, Cerebral energy metabolism

Stroke is the second leading cause of death worldwide. In recent years, we have learned a tremendous amount of knowledge regarding the injury mechanisms of strokes and identified numerous targets for therapeutic purposes. However, there is still extremely limited pharmacologic treatment for ischemic stroke. Currently there is thromobolytic treatment and mechanical embolectomy. Recombinant tissue plasminogen activator (rt-PA or alteplase) treatment for acute stroke must be given within 3 hours of onset and is given to only 2–3% of all acute ischemic stroke patients (Weintraub, 2006). The recently FDA-approved the MERCI devices for intra-arterial clot removal in ischemic stroke can be used at 8 hours after stroke, especially for patients ineligible for intravenous rt-PA (Katz and Gobin, 2006; Vora et al., 2008). Recanalization rate using MERCI retriever can reach 48%.(Smith et al., 2005) Another recanalization treatment using Penumbra Aspiration Device has also passed its Premarket Approval from the FDA (http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfPMN/pmn.cfm?ID=25864). How the beneficial effect of mechanical clot retrieval on improving patient clinical outcomes remains to be established.

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While stroke investigators have achieved progress in attempting to improve recanalization for clot-occluded intracranial arteries, the search for an effective neuroprotectant for acute stroke treatment has not been successful. In an extensive review of 1026 experimental treatments that have been tested, neuroprotective efficacy was superior to control conditions in 62% of the preclinical models of focal ischemia, in 70% of preclinical models of global ischemia, and in 74% of culture models. Of these experimental treatments, 114 have been tested in human with little to no success (O'Collins et al., 2006). No neuroprotectives have been approved for clinical use in stroke.

The apparent failure of neuroprotection for acute ischemic stroke has caused a series of active discussions in both academic and industrial fields, which is fruitful and constructive in attempts to establish criteria for preclinical stroke studies. Since the Stroke Therapy Academic Industry Roundtable (STAIR) published its first criteria in 1999, quality of study design problems and inflation of reported efficacy of neuroprotectants have continued to be major issues in this field. Translational research of neuroprotection for ischemic stroke has reached its critical stage. A strategic reconsideration is very much needed to aid in the search for new solutions. Going beyond the STAIR criteria, this article proposes strategic opinions for neuroprotection in ischemic stroke by reviewing

research work on major factors that determine the fate of ischemic tissue.

The STAIR criteria and the quality issue in neuroprotection validating system

The translational failure of neuroprotectants for acute ischemic stroke treatment has led to an investigation into the validating system for the efficacy of neuroprotective candidates. Many factors have been discussed as possible reasons for why experimental evidence of efficacy has not translated into efficacy in clinical settings. Some of the most commonly raised possibilities are species differences, inappropriate time windows, ineffective drug levels, inability of drugs to cross the blood-brain-barrier (BBB), use of young animals without co-morbidity, failure to model white matter damage, and heterogeneity of stroke subtypes in patients (Stroke Therapy Academic Industry Roundtable, 1999).To date, there have been five versions of Stroke Therapy Academic Industry Roundtable (STAIR) criteria. (Fisher, 2003; Fisher et al., 2007; Fisher, 2005; Stroke Therapy Academic Industry Roundtable, 1999; Stroke Therapy Academic Industry Roundtable, 2001) These STAIR standards, if followed strictly among investigators, likely could have minimized false positive conclusion from preclinical stroke trials.

Although the STAIR standards have been published for several years, the design quality issue in experimental stroke research is still a concern. (Dirnagl, 2006; Savitz, 2007; van der Worp et al., 2005). According to a scored-analysis of published data, many preclinical trials were carried out with lower than average quality. (van der Worp et al., 2005) A revisit of the preclinical data for the recently failed Stroke–Acute Ischemic NXY Treatment II (SAINT II) trial revealed serious quality issues. (Savitz, 2007)

Does STAIR help with increasing true positive discoveries?

The STAIR recommendations, if followed, could have tackled the problems in the validating system. The effective implementation of STAIR standards may reduce the inflated efficacy of tested neuroprotectants, in other words, the false positive results; it wouldn't necessarily improve the chance of a true positive discovery of an effective neuroprotectant for treating ischemic stroke. It is interesting that the FDA-approved effective recanalization treatments, including rt-PA treatment, MERCI device and Penumbra System, have passed through the same validating system whilst all the neuroprotectives failed their clinical trials. Most likely, the major problem of stroke treatment translation may hide in the early stage of translational research in identifying therapeutic targets, rather than in the late stage of validating therapeutics. So there is a need to reconsider our strategies in finding an effective neuroprotectant for acute ischemic stroke treatment. A basic understanding of the major factors that determine ischemic tissue's fate will be critical in identifying therapeutic targets. Successful recanalization therapies must also have significant effect on some of these major factors, so that they can improve the outcome of stroke patients.

Cerebral blood flow can determine the fate of ischemic tissue

Ample evidence supports the concept that blood flow is one major determinant of the fate of ischemic tissue. A given change in level of blood flow leads to a specific metabolic alteration and pathological outcome. Many thresholds for such changes have been clearly defined. For example, protein synthesis inhibition occurs when cerebral blood flow (CBF) is reduced to 55 ml/100 g/min (Hata et al., 2000; Hossmann, 1994); glycolysis becomes the main form of glucose metabolism when CBF reaches 35 ml/100 g/min; neurotransmitter disturbance begins at a CBF of 20 ml/100 g/min; and the ischemic core, with ATP depletion and anoxic depolarization, forms at a CBF below 15 ml/100 g/min.

Recent clinical studies that used computed tomography perfusion-derived CBF and cerebral blood volume plus magnetic resonance imaging (MRI) obtained CBF threshold levels for infarct (9–15 ml/100 g/min) and for penumbra (15–25 ml/100 g/min) (Murphy et al., 2006; Ohashi et al., 2005) that agree with those from previous studies. Dynamic imaging also demonstrated that recanalization salvaged penumbra in acute ischemic stroke. Another recently reported clinical study (Bardutzky et al., 2007) used MRI perfusion-diffusion mismatch technology to show that core and mismatch pixels that were ultimately salvaged had persistently higher CBF values during ischemia than non-salvaged regions, and that the severity of CBF reduction during ischemia seemed to be the main factor determining tissue fate. rt-PA treatment is effective because it restores CBF to the ischemic tissue, thus improving its fate. For a neuroprotectant to be effective, it must also act on a major factor that determines the fate of ischemic brain cells.

Energy metabolism as determinant of the fate of ischemic cells

After cerebral ischemia, there is early metabolic alteration. Focal cerebral ischemia results in an en-

ergy-depleted ischemic core and a penumbral zone with compromised energy metabolism.

Disturbed energy metabolism in the penumbra leads to a series of pathophysiological cascades, which, in turn, worsen energy metabolism. Many known pathophysiological processes that follow ischemia are able to make the compromised energy state even worse (Anderson et al., 1999; Swanson et al., 1997). These multiple vicious cycles contribute to the evolution of cerebral infarction. Many classic neuroprotectants are membrane stabilizers, which preserve intracellular ATP levels (Weigl et al., 2005). Accumulating evidence suggests that energy state, or the ATP level, determines the fate of ischemic tissue and the pathways for cell survival and cell death. Energy status has been shown to correlate well with cell survival and recovery in rat in vitro models of ischemia (Galeffi et al., 2000) and hypoxia (Wang et al., 2000) in hippocampal slice cultures, primary cortical astrocytic cultures (Yu et al., 2002), and in kidney tubule cells (Lieberthal et al., 1998). Differential ATP levels also determine the pathway to apoptosis. Evidence suggests that cells begin to enter the apoptotic pathway when ATP levels decrease to 25–70% of control levels and become necrotic when ATP levels are less than 15% of control. (Eguchi et al., 1997; Leist et al., 1997; Lieberthal et al., 1998; Nicotera and Leist, 1997; Nicotera et al., 1998). Thus, the energy state determines the fate of ischemic cells, and the fate of ischemic cells in the penumbra determines the evolution of the infarct.

Recanalization therapies performed within a limited time window are effective in saving ischemic tissue.(Rivers et al., 2006; Thomassen and Bakke, 2007) The recovery of energy metabolism of ischemic tissue following reperfusion (Paschen et al., 2000) strongly supports energy state being one of the determing factors for the fate of ischemic tissue although there are regional differences in energy recovery(Kuroiwa et al., 2000) and risks for secondary energy failure and reperfusion-related injury.(Lust et al., 2002)

The shortage of in-depth studies on penumbral energetics in experimental stroke.

Investigators may be under the impression that contemporary medical science has discovered all there are to know about energy metabolism. But the fact is that we know relatively little about the details of bioenergetics in the penumbral zone. Combining the search terms "Brain Ischemia"[MAJR] AND "Energy Metabolism"[MAJR] retrieves 466 publications on PubMed, which is 1.07% of the 43,446 articles retrievable on the topic of brain ischemia (search date 05-11-08). Of the 466 articles related to energy metabolism, 51 include focal ischemia, 125 include forebrain ischemia, and 53 include global ischemia, but only 12 mention the word “penumbra,” and only 2 or 3 use the terms “peri-infarct” or “boundary zone.” Due to technical limitations, previous research on energy metabolism of ischemic stroke has been struggling at the level of the substrate, such as glucose (Chang et al., 1998; Katayama et al., 1998), fructose-1,6-bisphosphate (Kaakinen et al., 2006), pyruvate (Yi et al., 2007), succinate (Pomytkin and Semenova, 2005), citrate (Mack et al., 2006), nicotinamide (Yang et al., 2002), ubiquinone (Tsukahara et al., 1999), oxygen (Rogatsky et al., 2003; Singhal, 2007), and hydroxybutyrate (Ottani et al., 2003; Ottani et al., 2004; Vergoni et al., 2000). Perhaps it is complacency that has partially caused the reluctance of stroke researchers to address penumbral energetics, the fundamental issue in acute stroke. Recent advances in energetics, MRI, optical imaging, and molecular biology have made it feasible to do systematic and in-depth energetic studies of acute ischemic strokes. A breakthrough in the strategies for improving the energy state of ischemic tissue will likely lead to the discovery of an effective neuroprotectant for acute ischemic stroke.

There is still hope for neuroprotection

The almost two decades of continued repeated failures on neuroprotection for acute ischemic stroke has disappointed both academic field and industrial field. Pharmaceutical companies lost huge investments and currently consider neuroprotection for ischemic stroke as an unreachable goal. A pessimistic feeling also exists in the academic field. Some authors even challenge the validity of using animal models for medical research (Fisher and Tatlisumak, 2005; Shuaib et al., 2007). However, the existing problems in preclinical studies indicate an urgent need to improve the quality of experimental stroke research, rather than a challenge against its value to human health and disease treatment.

A most recent article stated that neuroprotection without reperfusion may not be possible until we have innovative concepts in protecting ischemic neuronal injury. (Rother, 2008) This again demonstrated the urgent need for a reconsideration of our strategies for neuroprotection for acute ischemic stroke treatment. As pointed out by other investigators (Donnan and Davis, 2008; Hussain and Shuaib, 2008; Shuaib and Hussain, 2008), research on neuroprotection is still possible and we should continue in searching for an effective neuroprotectant. But where are the correct directions and what could be a better concept for

neuroprotection? We believe the following aspects in neuroprotection need to be strengthened, which may have the potential to bring forth a scientific breakthrough in this field.

1. More extensive and systematic studies of penumbral energetics

To find an effective means to preserve and/or restore penumbral energy state, we need to have a profound understanding of penumbral energetics, which will include steps from energy generation to consumption, transportation (Dzeja and Terzic, 2003), and signaling in neurons, glia, and endothelial cells at different anatomic, cellular, and subcellular locations, and in different time frames. The reality is quite complex. For example, under physiological concentrations, neurons prefer lactate for oxidative metabolism (Bouzier-Sore et al., 2006), but it is glycolysis, NOT lactate oxidation, that delays the occurrence of the anoxic depolarization (Allen et al., 2005). Furthermore, astrocytes have intracellular spatial differences in energy metabolism for the usage of oxygen (Hertz et al., 2007). Obtaining a complete understanding of penumbral energetics will likely require multi-disciplined, multi-centered collaboration.

Ascertaining the relationship between ischemic energetics and subsequent changes in genetics, proteomics, metabolomics, and lipidomics will be worth a systematic profiling. Many vicious cycles that cause energy status to deteriorate occur in penumbral tissue (Anderson et al., 1999; Swanson et al., 1997). Few genetic studies have been carried out in regard to acute experimental ischemic strokes. It was reported that global and focal cerebral ischemia induce many immediate early genes, such as c-fos, fos-B, c-jun, jun-B, jun-D, zif/268, Krox 20, and nur/77 (Akins et al., 1996). In the peri-infarct zone of a permanent focal ischemia model, 328 of 8740 genes on a microarray were found to be regulated by ischemia (Lu et al., 2003). In the peripheral blood of stroke patients, 100 genes were regulated, mostly in polymorphonuclear cells (Sharp et al., 2007). Unfortunately, those studies did not correlate their results with either penumbral energy state or the fate of ischemic tissue. With many molecular changes being identified in penumbral zone (Sharp et al., 2000), a systematic profiling of ischemic penumbra using technologies of proteomics, metabolomics, or lipidomics will aid in achieving a more complete understanding of the ischemic penumbra.

2. Rigorous study on the process of ischemic cell death

The process of ischemic cell death is still far from being fully understood. Other types of ischemic cell death may exist consequent to ischemia besides necrosis and apoptosis (Adhami et al., 2007). Evidence supports the possibility that there may not be a clean-cut type of cell death and that even necrosis may be a variety of programmed cell death (Festjens et al., 2006; Golstein and Kroemer, 2007). A new theory is forming toward a uniform requirement of energy in different forms of cell death (Chiarugi, 2005). Energy state seems to play an important role in the connection between different types of cell deaths (Chiarugi, 2005; Skulachev, 2006; Zong and Thompson, 2006). Extensive research on the forms and processes of ischemic cell death may lead to discoveries of new determining factors that control and differentiate ischemic cell death, facilitating the discovery of an effective neuroprotectant for ischemic strokes.

3. Use of tissue and intracellular ATP levels as parameters for prescreening of neuroprotectants.

Because energy state determines the fate of ischemic tissue and cells (Chiarugi, 2005; Skulachev, 2006), it may be considered as important as regional CBF for the outcome of ischemic tissue, and therefore may be helpful if adopted as a basic parameter for the study of neuroprotection. As has been seen from past failures, if a treatment can't stabilize, improve, and restore the energy state of ischemic tissue or cells, it will likely be ineffective at protecting neurons from ischemic damage.

4. Improve the methodology for patient screening and the potential use of ATP imaging for the identification of penumbra.

Appropriate selection of patients is very important for treatment and for clinical trials of potential neuroprotectants. Patient selection has a big impact on the efficient use of limited medical resources as well as on the efficacy and sensitivity of clinical trials. Currently, Diffusion-weighted imaging (DWI) and perfusion-weighted imaging (PWI) MRI studies are the most widely used clinical imaging modalities to detect the presence of penumbra (Guadagno et al., 2004; Lovblad et al., 2003). The PWI–DWI mismatch region, defined as the difference in volume of tissue between the smaller diffusion lesion and the larger perfusion lesion is thought to approximate the ischemic penumbra. However, use of PWI–DWI mismatch for the prediction of salvageable tissue after acute ischemic strokes has many limitations and problems (Kidwell et al., 2003). A recent study showed no clear association between the presence of a DWI–PWI mismatch and lesion expansion, casting doubt on the value of the DWI–PWI mismatch as

a clinical marker of tissue-at-risk (Rivers et al., 2006). Approximately half of all patients without DWI–PWI mismatch have lesion growth and may benefit from acute stroke treatment; these patients probably should not be excluded from clinical trials (Rivers et al., 2006). A better approach for predicting the salvageable ischemic tissue is urgently needed to guide the clinical decision to use thrombolytic therapy. Echo-planar spectroscopic imaging (EPSI) (Ulrich et al., 2007) and optical ATP imaging (Bell et al., 2007) are new technologies that may prove useful for this purpose.

5. Pharmacological preconditioning.

It is possible that ischemic preconditioning occurs in patients who have undergone transient ischemic attacks (TIA) (Schaller, 2005; Wegener et al., 2004), but TIAs do not naturally and effectively induce ischemic tolerance (Johnston, 2004). Some neuroprotectants may be effective if used before ischemic insults as a form of pharmacological preconditioning for ischemic injury of brain (Brambrink et al., 2004) and spinal cord (Caparrelli et al., 2002). Patients who have experienced TIAs or previous strokes carry a risk of recurrent strokes between 5 and 20% per year (Grau et al., 2001; Johnston et al., 2007; Weimar et al., 2002; Wilterdink and Easton, 1992). Aspirin, with or without dipyridamole, can be effective for secondary prevention of ischemic strokes in such patients, with up to an 18% or 37% respective reduction in stroke risk. (Diener et al., 1996) In other words, the majority of these high-risk patients will suffer ischemic strokes despite being treated with antiplatelet medications. An effective pharmacological preconditioning protocol could be used as an alternative way to reduce ischemic injury.

6. Metabolic suppression and energy delivery

Because energy state is one of the determining factors that controls and differentiates ischemic cell death, improvement in energy state of the ischemic tissue can be achieved only through providing extra energy or reducing the demand and consumption of energy. Liposome encapsulated adenosine triphosphate (ATP)(Arakawa et al., 1998) can be used for direct energy delivery and has shown a protective effect in intestinal injury from hemorrhagic shock (Zakaria el et al., 2005) and skin wound healing (Chiang et al., 2007). Because of the enriched purinergic receptors in nerve cells, direct energy delivery has not been successful in the nervous system. More research will be needed to bypass purinergic receptors (Boucsein et al., 2003; Chen et al., 2007; Siow et al., 2005).

Hypothermia has been proved an effective means for neuroprotection in animal models. (Hammer and Krieger, 2003; Konstas et al., 2006) However, its clinical application has encountered many serious complications such as severe coagulopathy, cardiac failure, and uncontrollable intracranial hypertension, which cause a high mortality.(Jian et al., 2003; Schwab et al., 2001) More work is needed to optimize protocols for the rewarming process and to develop effective protection against hypothermia-related severe side-effects.

Progress in the study of hibernation has revealed a promising approach for metabolic suppression. During the torpor state of hibernation, the metabolic rate can be reduced to as low as 2% of baseline level (Geiser, 1988), while CBF can be reduced to 11% of baseline (Frerichs et al., 1994) without causing injury. Hibernation provides ischemic tolerance even in hippocampal slices obtained from hibernating animals (Ross et al., 2006). Neuroprotection by massive metabolic suppression under a controlled torpid state could be highly valuable to translational stroke research (Drew et al., 2007; Fisher and Henninger, 2007; Frerichs, 1999; Lee and Hallenbeck, 2006).

Conclusion

The translational failure in finding an effective treatment for ischemic strokes necessitates a strategic reconsideration for experimental stroke research. More research efforts and support should be diverted to the subfield of ischemic energetics so that a breakthrough in this subfield could occur in the near future. It is likely that manipulating intracellular energy state with novel approaches, pharmacological preconditioning, and controlled torpid state may facilitate the discovery of an effective treatment for ischemic strokes.

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