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TBI PHARMACEUTICALS — The Long Odyssey of Cyclosporine is Almost Over

NeuroVive Pharma updates on cyclosporine's progress toward approval as a pharmaceutical for treating moderate to severe traumatic brain injury

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Cyclosporine is currently in phase II clinical trials for TBI. The aim of this review article is to provide an overview of the most relevant studies outlining cyclosporine's potential as a TBI pharmaceutical.

INTRODUCTION

Impressive advances in acute-care treatment of traumatic brain injuries (TBI) over the past few decades have led to increased survival rates, and also have led to rising costs associated with providing long-term care to the physically and mentally disabled. In addition, over the past decade, the silent epidemic of TBI has come to the fore as tens of thousands of wounded American and allied soldiers return home from the Middle East, suffering hidden or visible TBIs and trauma caused by blast injuries from improvised roadside explosions.[1]

Due to the economic and social costs of TBI, a significant ongoing international effort is underway to develop and apply emerging new clinical and preclinical pharmaceuticals that offer potential in post-injury medical treatment to mitigate the cascading additional brain damage that occurs during the critical secondary phase in TBIs. Among these is an interesting pharmaceutical compound called cyclosporine (also known as cyclosporin-A, or CsA) that has been found to have both significant neuroprotective capabilities and the ability to moderate the resulting damage and long-term disability associated withTBI.[2,3,4,5]

What is the potential for cyclosporine? Preclinical mouse model studies show an 80% reduction in neural damage through the application of this pharmaceutical.[6,7] Almost two decades in development for neuroprotection, researchers have recently made significant advances toward gaining approval for CsA as a treatment to greatly ameliorate the effects of moderate to severe TBI in humans by reducing the amount of brain cell death during the all-important secondary stage of TBI characterized by cascading waves of biochemical imbalances that continue to inflict new damage.

TWO STAGES

There are two stages that occur in traumatic brain injuries. The first takes place at the time of injury (due to a gunshot, blast, fall or hit, for example). This initial stage could be either a closed-head injury or an open wound, and medical emergency personnel focus on treating the wound or injury and, importantly, stabilizing the patient's vital functions.

The second stage of damage to the brain takes place after the initial insult, as the injury continues to develop and worsen in the hours and days following the initial trauma. In this secondary stage, the trauma to the brain triggers a series of cascading intra-cellular biochemical reactions that end up causing severe demise of brain cells, brain damage and expanded disability. If this secondary stage can be mitigated, the eventual damage and disability can be greatly reduced, enabling the victim to get closer to full recovery.

Some of the secondary-stage mechanisms believed by researchers to be involved in brain-cell death after TBI include uncontrolled release of signalling molecules (neurotransmitters), cellular calcium overload, inflammation, energy failure, oxidative damage, and the overactivation of enzymes such as calpains and caspases.[8]

All of these are believed to create the intra-cellular and extra-cellular conditions that lead to the destruction of millions of additional brain cells, and

in turn the extra damage and disability that occurs during the secondary stage. Many of these conditions are being targeted by a variety of pharmaceutical compounds and medical treatments (such as forcing oxygen into the brain through the use of hyperbaric chambers) that are in various stages of clinical development.[9] However, by targeting the protection of mitochondria inside brain cells, cyclosporine has emerged as perhaps the most promising of these.

CENTRAL ROLE OF MITOCHONDRIA IN TBI

Research confirms that mitochondria — the cellular energy (ATP) producers inside the brain cells — play a central, pivotal role in neuronal cell death or survival; mitochondrial dysfunction is in fact considered an early event that causes neuronal cell death in brain injury situations. The uncontrolled release of signalling molecules, with resulting overstimulation/stress of brain cells and accumulation of high levels of intracellular calcium, may be the initial major mechanism that leads to neuronal cell death.[10]

How does this affect brain cells? Increases in calcium lead to its rapid uptake into the mitochondria (which act as cellular sinks for calcium). However, the excessive transport and uptake of calcium will negatively impact mitochondrial energy production given that the driving force for both ATP production and calcium transport relies on the "proton motive force" (the proton gradient created over the mitochondrial inner membrane by the respiratory chain). Further, excessive calcium uptake by mitochondria, in combination with energy failure, leads to the formation of protein channels (pores) in the inner membrane — the induction of the so-called mitochondrial permeability transition (mPT) pore.

The increased permeability of the inner membrane caused by the mPT pores immediately collapses mitochondrial function and structure (that is, when the pores are opened, the osmotically active inner compartment (matrix) of the mitochondria will attract water, and the mitochondria will swell and pop like balloons). In addition to causing the cessation of energy production, upon induction of the mPT, the stored calcium and harmful proteins will be released from mitochondria, resulting in an avalanche of further mitochondrial collapse, cellular energy depletion, and subsequent cell death. When brain-cell death is repeated millions of times during the cascading biochemical imbalances that characterize the secondary phase, the extent of brain damage and eventual disability is greatly increased.[11]

Protecting the mitochondria by targeting the mPT is a viable neuroprotective approach that has emerged over the last decade. Published research has found that the protein cyclophilin D is an essential component to opening the mPT pores,[12] and that cyclosporine binds to cyclophilin D and inhibits the induction of mPT.[13] The result of this inhibition is that mitochondria can absorb much more calcium without collapsing, enabling them to survive. As mitochondria survive to produce energy for the brain cell, fewer brain cells die during the secondary stage of the TBI. Protecting brain cell mitochondria and energy production is the critical front line in the war against TBIs.

CYCLOSPORINE PROTECTS

Cyclosporine was discovered in 1969 when it was first isolated from the fungus Tolypcladium inflatum in Norway by researchers working for Sandoz (now Novartis). Its impressive immunosuppressive properties led it to become a pharmaceutical to prevent tissue rejection in organ-transplant recipients. It has been in use for immunosuppressive applications since the early 1980s as a commercially successful Novartis product called Sandimmune®.[14]

CsA's ability to protect the mitochondria in

the brain by binding to cyclophilin D and preventing the induction of the mPT was first discovered in 1993–94, during which time the co-author (Eskil Elmér), along with his Japanese colleague Hiroyuki Uchino, were conducting experiments in cell transplantation. An unintended finding was that CsA was strongly neuroprotective when it crossed the blood–brain barrier.[15] The discovery became the starting point for basic research and patent applications in this promising new avenue of neuroprotection. These activities have expanded, and continue to the present day.

The research mapping out CsA's extensive neuroprotective capabilities has been running continuously since 1993, and many independent international research teams have since conducted and published numerous studies confirming that CsA is a powerful nerve-cell protector in TBI, stroke, and brain damage associated with cardiac arrest. Advanced studies show that CsA is also able to protect mitochondria in heart tissue facing reperfusion injury during heart attacks, as it inhibits the actions of cyclophilin as part of the intracellular biochemical imbalances that often accompany reperfusion of a blocked artery.[16]

Patents were applied for this work of developing cyclosporine-based products for acute conditions and diseases affecting the brain. In 1999, the U.S. patent was approved and, in 2000, the CsA product name NeuroSTAT® was registered. Later, the patent portfolio around CsA's impact on the CNS and other areas was expanded greatly under NeuroVive Pharmaceutical AB (Sweden).

Today, NeuroVive's NeuroSTAT version of cyclosporine is a fully developed and finalized product. An important advancement in NeuroSTAT over other cyclosporine-based pharmaceuticals is that its formulation is made using a patented non-allergenic lipid emulsion to keep the lipophilic CsA drug in solution.

ACCELERATING PROGRESS

It's been almost two decades since cyclosporine's neuroprotective capabilities were first discovered, and there is still some way to go. However, CsA's promise as a TBI pharmaceutical continues to develop, with full commercialization for NeuroSTAT now in sight.

In 2010, NeuroSTAT received orphan drug status, both from the U.S. FDA and in Europe, for the treatment of moderate to severe TBI. In March 2011, NeuroVive announced it would be working with the European Brain Injury Consortium to conduct a phase II/III adaptive study on NeuroSTAT.[17] These clinical trials should provide the basis for the registration of NeuroSTAT in Europe, and possibly the U.S. and elsewhere. U.S.-based clinical trials are also being planned, and NeuroVive is working with a partner in China to develop similar trials.

Of course, the challenge of such an effort (i.e., no TBI pharmaceutical has ever been approved and more than 30 TBI pharmaceuticals have previously failed phase III studies) is to translate the research results into clinical benefits in patients, and be able to recruit sufficient patients within a reasonable time frame.

CYCLOSPORINE ALREADY SHOWN TO PROTECT HUMAN MITOCHONDRIA

Against this backdrop of many failures costing millions of dollars, NeuroSTAT stands out with much more positive potential for approval. First, cyclosporine has already been in use in humans since the 1980s, and is a well-established pharmaceutical compound in medicine. Second, and most important, cyclosporine has already been shown to have

a significant positive protective effect on human mitochondria in a small group study published in the *New England Journal of Medicine* (2008) on reducing reperfusion injury in myocardial infarction. In this study, cyclosporine delivered a 40 percent reduction in infarct size by protecting heart tissue mitochondria from the cascading biochemical imbalances caused by re-establishing blood flow through the blocked artery (called reperfusion).[18] Since similar pathogenic mechanisms are suggested in neuronal tissue in human TBI, those exciting results are very promising. In addition, it has been confirmed that isolated human brain mitochondria display the same calcium-induced mPT, that the target cyclophilin D exists in human brain tissue, and that inhibition of cyclophilin can protect human brain mitochondria.[19]

In that same issue of the *NEJM*, the journal called for more extensive studies to confirm cyclosporine's ability to reduce reperfusion injury.[20] To that end, CicloMulsion®, the cardiac reperfusion injury version of NeuroVive's NeuroSTAT pharmaceutical (the CicloMulsion product formulation is identical to NeuroSTAT) is currently undergoing a 1,000-patient, investigator-initiated phase III study in Europe. With the first patient enrolled in April 2011, this independent study aimed at confirming cyclosporine's ability to ameliorate reperfusion injury has already enrolled hundreds of patients. Results of this pivotal trial are expected in 2014.

Back in neuroprotection, international research groups continue to study various aspects of cyclosporine's ability to moderate the negative effects of TBI. For example, the National Institute of Neurological Disorders and Stroke (NINDS) of the U.S. National Institutes of Health recently granted $6.7 million to pediatric TBI researchers at the University of Pennsylvania for a preclinical porcine TBI study. The following was published in a university news story:

"Because Cyclosporin A [sic] has a safety profile in children and is in therapeutic use or clinical trials for other indications and has a treatment record for neuronal injury in rodents, the scientists hope to move quickly from this preclinical trial to an FDA-approved clinical trial for children with TBI. Successful trials may bring the first pharmacological treatment to rescue function and promote longer-term neurological recovery in some of the hundreds of thousands of children who suffer from traumatic brain injuries every year."[21]

SAFETY CONCERNS ADDRESSED

Traditionally, there have been two potential side effects of using cyclosporine, both of which have been addressed through the new formulation. NeuroSTAT uses a non-allergenic lipid emulsion to keep CsA as a lipophilic drug in solution. This is an improvement on the traditional use of cremophor and ethanol after they were found to cause serious anaphylactic reactions in some patients (about one per 1,000 transplantation patients who, as a part of the immunosuppressive regime, are also medicated with steroids).

The other concern with the use of this immunosuppressant is that it could lead to opportunistic infections taking hold in TBI patients. However, since it's also only used for a few days, and since the acute care of TBIs often includes antibiotics, the chances of CsA fostering bacterial infections are low. In addition, it turns out that cyclosporine affects a different part of the immune system. As one recent 2012 review article of neuroprotective pharmacology therapies in TBI noted, "Ciclosporin [sic] has been investigated for use in TBI through several phase II clinical trials. Thus far, these trials have demonstrated that ciclosporin is safe in the TBI population...."

The article concludes its CsA section by noting that "Ciclosporin appears to have little effects [sic] on T-lymphocyte counts or incidence of infection in the acute phases of TBI."[22]

MITOCHONDRIA, CSA AND OTHER NEURODEGENERATIVE DISEASES

Aside from acute injuries like TBI, cardiac reperfusion injury and stroke, mitochondrial dysfunction due to calcium overload and the resulting cyclophilin involvement in the construction of the mPT pore have been implicated in the genesis or progression of neurodegenerative diseases or conditions such as ALS, Alzheimer's, Parkinson's, Huntington's, epilepsy, MS, autism, chronic fatigue syndrome and others.[23, 24, 25] Cyclosporine's ability to inhibit cyclophilin and protect mitochondria from destruction or dysfunction is being studied in a number of these medical conditions. In Alzheimer's, for example, a recent study from the University of Rochester published in Nature in 2012 demonstrated that researchers were able to show that an excess of cyclophilin A led to Alzheimer's-like brain damage symptoms in mice, reduced blood flow to the brain, and an increase in the presence of toxic Alzheimer's precursor substances. The administration of cyclosporine inhibited cyclophilin's actions and the Alzheimer's symptoms were reversed.[26]

Mitochondrial dysfunction may also play a significant role in cardiovascular diseases, diabetes, cancer and general aging. Intracellular calcium overload may be the trigger for these conditions, and the resulting cyclophilins and construction of mPT pores may play a critical role in promoting less-than-optimal mitochondrial performance and cellular functioning across a broad spectrum of disorders.[27, 28] As the most advanced cyclophilin inhibitor available, cyclosporine and its emerging next-generation derivatives hold great potential to address a number of these neurodegenerative and cardiovascular conditions as this compound's pharmaceutical formulations continue on track toward becoming the first clinically proven mitochondrial medicine.

CONCLUSION

Looking ahead, NeuroVive continues to research and develop next-generation variants of cyclosporine-based cyclophilin inhibitors that are not immunosuppressive and are able to cross the blood–brain barrier to deal with TBI and also stroke. While it may take some time to move these into clinical trials, the special formulation of cyclosporine in NeuroSTAT as a first-generation cyclophilin inhibitor is a fully formed product ready for application in moderate to severe TBI once its clinical trials are complete.

Assuming all goes according to plan, cyclosporine's early promise from its serendipitous discovery as a neuroprotectant in the 1990s could be fulfilled within the next three to five years, giving neurologists and neurosurgeons worldwide an exciting new pharmaceutical to treat the silent epidemic of traumatic brain injuries.

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