BUDDHA BIOPHARMA, LTD.

Dicholine Succinate Treatment for Alzheimer's Disease and Other Neurodegenerative Disorders

Alzheimer's Disease

Alzheimer's disease (Alzheimer's) is a progressive and ultimately fatal neurodegenerative disorder characterized by multiple cognitive deficits, including the progressive impairment of daily living activities, and a variety of neuropsychiatric symptoms and behavioral disturbances. In 2006 it was estimated that the prevalence of Alzheimer's exceeded 26 million cases worldwide, and this is expected to continue to increase significantly with the growing elderly population. The percentage of persons with Alzheimer's currently increases by a factor of two approximately every five years of age, meaning that 1 percent of 60-year-olds, and about 30 percent of 85-year-olds, have the disease. The total worldwide cost for dementia-related care has been estimated (in 2005) to be US $315 billion annually (for reference, the worldwide pharmaceutical market was estimated to total about $480 billion annually in 2005) [1]. In developed countries, Alzheimer's is one of the costliest diseases to society

There is no known cure for Alzheimer's. Some available treatments do deliver some symptomatic benefits, but these remain palliative in nature. Currently over 1000 clinical trials of Alzheimer's related drugs have either been completed or are in progress [1], but to date just four acetylcholine esterase inhibitors and one N-methyl d-aspartate receptor antagonist have been approved as effective for the symptomatic treatment of Alzheimer's. Even these drugs are not effective in every patient, and lose efficacy over time. Thus there is a pressing need for new and effective therapies—and for new therapeutic approaches—to fill the vacuum.

Etiology of the Disease: Competing Hypotheses

The causes of Alzheimer's and related neurodegenerative diseases are as yet not well understood. The current trend in Alzheimer's research is to view the pathogenic process as multi-faceted, with no single identifiable cause.

Competing hypotheses regarding the etiology of Alzheimer's include:

- The once dominant *amyloid cascade* hypothesis, which holds that the production and accumulation of *β*-amyloid (A*β*) plaques is central to the pathogenesis of Alzheimer's disease [2]. The pathological amyloid cascade progresses from the generation of the *β*-amyloid peptide from the transmembrane amyloid precursor protein (APP), through multiple secondary stages, to eventual cell death. Accordingly, anti-amyloid treatment

  was once widely considered to be the most promising approach for the prevention and treatment of Alzheimer's. However, a 2004 study [3] found that deposition of A*β* plaques correlated only weakly with neuron loss in Alzheimer's patients; and in a 2008 study [4] an experimental vaccine was found to clear the A*β* plaques in human trials, but this was

not determined have any significant effect on dementia. To date, all AD clinical trials based on A*β* as a therapeutic target have failed.

- The *modified amyloid* hypothesis, which suggests that a close relative of the A*β* protein may be a significant cause of the disease. In this approach, it is conjectured that an amyloid-related mechanism that modifies neuronal connections in the brain in early development is triggered pathologically as part of the aging process, contributing to the neurodegeneration underlying Alzheimer's [5]. Here N-APP, a fragment of the A*β* precursor protein (APP), is cleaved from APP by the same class of enzymes. N-APP triggers the self-destruct pathway by binding to the neuronal “death receptor” DR6. No therapeutic strategies suggested by this model have so far been proven effective in the treatment of Alzheimer’s.

- The closely related *tau hypothesis*, which associates the progression of Alzheimer’s with the formation of neurofibrillary tangles [6,7]. The formation of such tangles is attributed to the hyperphosphorylation and consequent misfolding of microtubule-associated *tau proteins*. One of the tau proteins’ main functions is to modulate the stability of axonal microtubules. In this model, hyperphosphorylated tau couples with normal tau, resulting in the formation of self-assembling neurofibrillary tangles inside neurons. This is thought to cause the microtubules that are normally stabilized by the tau proteins to disintegrate, disrupting the neuron's transport system, eventually resulting in cell death. While specific pharmacological targets suggested by this approach have been discussed [8], no treatment for Alzheimer’s based on the tau model has yet been proven effective.

- The *insulin resistance* hypothesis, which attributes the progression of Alzheimer’s to dysfunctional insulin/lGF-1 receptor signaling [9,10]. There is substantial evidence that dysfunctional insulin/lGF-1 receptor signaling is associated with the neurodegenerative progression of Alzheimer’s. In this model, impairments in brain insulin/IGF-1 signaling lead to increased expression of APP and accumulation of APP-A*β*. Insulin activates clearance of *β*-amyloid peptides from the brain by enhancing the transport of APP and *β*-amyloid from neurons, and by accelerating the degradation of amyloid plaques through the insulin degrading enzyme (IDE), whose genetic expression is also under the control of insulin. While therapeutic strategies have recently been suggested based on this model [11], and have recently been the subject of a pilot clinical trial that has produced encouraging results, none has yet been proven effective for the treatment of Alzheimer’s disease.

- The *mitochondrial* hypothesis, according to which early mitochondrial dysfunction causally precedes the pathological amyloid cascade [12,13]. In one A*β*-independent variant of this hypothesis, such mitochondrial dysfunction is caused by toxic products of apolipoprotein, and particularly apoE4, degradation: apoE4 proteolytic fragments target the mitochondria of neurons, leading to mitochondrial dysfunction and neurotoxicity. This has led to the suggestion of specific therapeutic targets for the treatment of Alzheimer’s

  [14]. However promising this kind of approach may currently appear to be, no effective therapeutic procedures based on this model that are able to ameliorate the deleterious effects of ApoE4 on mitochondrial function have so far become available.

Neuronal Insulin resistance and Insulin Receptor Sensitization

There is already abundant evidence supporting the theory that insulin acts in the brain to regulate neuronal glucose metabolism, and that insulin regulates cognitive functions such as learning and memory, feeding behavior (suppression of appetite), and reproduction [15]. The accumulated evidence suggests, in addition, that *neuronal insulin resistance* resulting from impairments to cerebral insulin receptor signaling may contribute significantly to age-related cognitive deficits and thus to neurodegenerative diseases such as Alzheimer's and Parkinson's. The enhancement of brain insulin receptor signaling therefore presents itself as a promising therapeutic strategy for the treatment of cognitive disorders resulting from age-related neurodegeneration.

It is already well known, based on hard empirical evidence, that autophosphorylation of the insulin receptors is the first critical step in the activation of neuronal insulin receptor signaling [16]. In this activation mechanism, which functions as a *negative feedback* loop, upon autophosphorylation receptor tyrosine kinase initiates the insulin signal transduction mechanism that mediates all biological activity of insulin in the brain.

Our own research [17] has in addition produced strong evidence that activation of the insulin receptor is actually controlled by a regulatory *double* negative feedback loop which in our model transforms grade inputs of insulin to activate the neuronal insulin receptors. In addition to the previously known elements of this activation feedback loop (receptor tyrosine kinases and cellular tyrosine phosphatases) we have discovered an additional systemic element, the neural mitochondrion. We have discovered that when activated in response to insulin binding to the mitochondrial receptors, mitochondrial respiration produces *nanomolar spikes of hydrogen peroxide* (H_2O_2)*,* and that this has the effect of inhibiting de-phosporylation of the neuronal insulin receptors by tyrosine phosphatases. We have also found empirically that this process depends on the concentration of *succinate*, a well-known respiratory substrate [18].

We therefore see great potential for the development of effective treatments of neuronal insulin resistance—for the improvement cognition in elderly patients, and for the treatment of various other age-related cognitive disorders related to neurodegeneration—based on new techniques of neuronal insulin sensitization that specifically target the brain.

Buddha's Molecular Approach

Accordingly, we have adopted a molecular paradigm for the treatment of Alzheimer's, working through specific effects on mitochondrial respiration in neurons.

The molecular basis of Buddha's technology is our discovery that the mitochondrial respiratory chain is involved in the early activation of the insulin receptors in neurons.

Based on our discovery that activation of the mitochondrial respiratory chain in neurons by insulin causally precedes the critical kinase autophosphorylation step of neuronal insulin receptor activation, we have concluded that it should be technically feasible to inhibit or enhance insulin action on neurons, by inhibiting or promoting the production of H_2O_2 by mitochondrial respiration.

More specifically, since we have found that the activation of the mitochondrial insulin receptors is itself dependent on concentrations of succinate, we are proposing to develop a treatment for neuronal insulin resistance, involving the intranasal administration of succinate, that is designed to specifically target neuronal insulin receptors in the brain for sensitization.

Since relatively large molecules cannot cross the blood-brain barrier, and are thus neither appropriate nor effective for insulin sensitization of neurons in the brain by intranasal administration, we propose the use of a relatively small molecular species for this purpose that is not subject such limitations. Also, ideally the selected compound would either be human endogenous, or would be expected to break down easily into stable human endogenous metabolites, in order that the risks of possible toxic side effects be minimized.

Dicholine Succinate

A compound meeting the above specifications that is capable of directly activating neuronal mitochondrial respiration, and thus sensitizing neuronal insulin receptors in the human brain by intranasal administration, has been identified, namely: Dicholine Succinate.

Dicholine Succinate is the only currently available insulin sensitizer that is capable of acting directly on neurons in the brain.

In addition, since choline and succinic acid are human endogenous, they are unlikely to result in adverse toxicological effects in human subjects at the concentration levels expected to be required for effective treatment.

Based on studies we have carried out with animal models of Alzheimer's disease, vascular dementia, and normal aging [18], we have already found this treatment to be effective in improving cognition (e.g. learning and memory). We have found in addition that the same mode of treatment improves neuron functioning (NMR *in vivo*); and that it also has the effect of up-regulating choline acetyltransferase, the key enzyme for biosynthesis of the neurotransmitter *acetylcholine*.

The A*β* Connection

Our research has also revealed links between the deleterious effects of ApoE4, dysfunctional insulin receptor signaling in the brain, and the amyloid cascade hypothesis.

There is strong experimental and clinical evidence that optimal insulin action in the brain is required for the prevention of *β*-amyloid accumulation in the brain, and that insulin accelerates the trafficking of amyloid precursor protein (APP) and *β*-amyloid peptides from neurons [19,20] and enhances the expression of the insulin degrading enzyme (IDE) responsible for *β*-amyloid degradation in the brain [21]. Such evidence will provide a sound empirical basis for the separate investigation of Dicholine Succinate, the neuronal insulin sensitizer, as a promising agent for anti-amyloid therapy.

The Russian Studies

In fact the effects of Dicholine Succinate have already been extensively investigated in Russia, and the following promising results have already been obtained in non-clinical studies conducted at various Russian research institutes:

- Dicholine Succinate significantly increases the effects of suboptimal insulin concentrations in a culture of rat neurons, in *in vitro* studies
- Dicholine Succinate significantly ameliorates cognitive deficits induced by normal aging in mice (in a normal aging model)
- Dicholine Succinate significantly ameliorates cognitive deficits induced by chronic cerebral hypo-perfusion in rats (two-vessel occlusion model of vascular dementia and Alzheimer's)
- Dicholine Succinate significantly ameliorates cognitive deficits induced by the administration of *β*-amyloid peptide 25-35 into the *nucleus basalis Magnocellularis* of rat brains (model of amyloid-induced Alzheimer's)
- Dicholine Succinate significantly ameliorates cognitive deficits induced by scopolamine, a muscarinic antagonist
- Dicholine Succinate significantly increases the N-acetylaspartate level in the brains of middle-aged mice, according to data of 1 H MRS in vivo, as compared to age-matched control
- Dicholine Succinate significantly increases the N-acetylaspartate level in the brains of rats with chronic cerebral hypoperfusion, according to data of 1 H MRS in vivo, as compared to sham-operated control
- Dicholine Succinate significantly increases the whole-brain level of Choline
- Acetyltransferase, the key enzyme of acetylcholine synthesis, was diminished by the injection of *β*-amyloid peptide 25-35 into the *nucleus basalis magnocellularis* of rat brains, as compared to a sham-operated control

Also in Russia, pre-clinical tests (toxicological studies, etc.) of Dicholine Succinate administered by injection have already been completed, and pre-clinical tests of Dicholine Succinate administered intranasally are also in progress.

Dicholine Succinate has already been licensed for commercialization as a therapeutic drug by a major Russian pharmaceutical company.

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