A Cell That Acts Like a Pill: Using iPSC-Derived Cholinergic Neurons to Treat Alzheimer’s Disease

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Abstract
Alzheimer’s disease (AD), the most common subtype of dementia, affects approximately 44 million people worldwide. One of the most major and direct causes of AD, as proposed by the cholinergic hypothesis, is a severe deficiency of cholinergic neurons located in the basal forebrain. This paper proposes a novel approach of using replacement therapy to treat Alzheimer’s disease with stem cell technology. The overarching idea is to inject new cholinergic neurons, which are developed from induced pluripotent stem cells (iPSC), into the brains of AD patients to reverse the cholinergic deficit outlined in the cholinergic hypothesis. The proposed idea is founded on a similar study carried out recently by a team lead by Jeffrey S. Schweitzer that used iPSCs to create dopaminergic neurons which were injected into the brain of a Parkinson’s disease patient and has been proven successful. The proposed treatment development plan would consist of three stages: development of induced pluripotent stem cells and differentiation into basal forebrain acetylcholinergic progenitor cells (APCs); animal studies where researchers inject APCs into the basal forebrain of AD mice models and track progress and improvement in cognitive behavior; and clinical trials involving volunteer AD patients when the procedure of APC development and injection will be repeated, and results will be analyzed. The resulting effects of this treatment can be expected to yield medical, economic, and social benefits.

Keywords: acetylcholine, Alzheimer’s disease, cholinergic, dementia, induced pluripotent stem cells, neurons, replacement therapy

1. Introduction
1.1 Alzheimer’s Disease
Alzheimer’s disease (AD) impairs memory, cognitive skills, and thought processing ability (Centers for Disease Control and Prevention [CDC], 2020). Symptoms worsen with the passage of time as AD is a progressive and neurodegenerative disease (Alzheimer’s Association, n.d.). AD weakens memories, through shrinkage of the hippocampus; thinking, communication, and reasoning skills, through shriveling of the cerebral cortex; and motor coordination as displayed in Figure 1 (CDC, 2020). The severity of symptoms depends on the stage of the victim’s disease. In the mild stages of Alzheimer’s, individuals may experience mild memory loss, confusion with speech, and delayed responses to everyday tasks. In the moderate stage of AD, individuals may experience problems with recognizing family members, reliance on assistance for walking and eating, and even hallucinations. In the severe stage of AD, individuals fully depend on palliative care and may spend most of their time in a sleeping state (National Institute on Aging [NIA], 2021). AD belongs to a family of dementias, such as Parkinson’s Disease, Huntington’s Disease, Lewy Body Dementia, and Vascular Dementia, and it is the most common out of all dementias accounting for 60-80% of all diagnoses (Alzheimer’s Association, n.d.). Contrary to popular belief, Alzheimer’s is not a natural result of aging, and individuals should receive proper treatment and care to ensure quality of life (Alzheimer Society, n.d.).
Figure 1. Artistic illustration comparing the brain of an Alzheimer’s patient to a healthy brain. Depicts sagittal section of normal brain (left) compared to AD brain (right) which shows shrinkage of brain volume and hippocampus along with enlargement of ventricles.

1.2 Impact of Disease

Alzheimer’s disease afflicts around 44 million people, and rising, worldwide, and researchers estimate that an AD diagnosis is given around the globe every three seconds (Alzheimer’s News Today, n.d.; BrightFocus Foundation, n.d.). The annual costs of treating the disease in the US may surpass $1.1 trillion by 2050, according to researchers, making AD ever more burdensome on the American economy (Alzheimer’s Association, 2021). However, despite research throughout the 20th and 21st centuries, scientists have still not uncovered a precise etiology of AD in order to develop a cure for the disease.

1.3 Hypotheses for Causes of Alzheimer’s Disease

A few hypotheses outline current known causes of Alzheimer’s, such as the amyloid hypothesis, tau hypothesis, and cholinergic hypothesis.

The amyloid hypothesis ascribes that a major cause of AD is the accumulation of beta-amyloid plaques in the brain, which trigger immune cells, causing inflammation and neuron death (Alzheimer’s Association, 2017). Beta-amyloids are produced as a result of the enzymatic splicing of activated amyloid precursor proteins (APP), which are found on the surface of neurons (Alzheimer’s Association, 2017). In a normal brain, these beta-amyloids are broken down and cleared away by microglial cells, but a brain affected by AD fails to do so (NIA, 2017). Resulting beta-amyloids cluster into fibrils, then beta-sheets, and finally, chemically stick together to form plaques. These beta-amyloid plaques build up outside neurons, blocking synapses and neuron communication, causing neurodegeneration (Alzheimer’s Association, 2017).

Similar to the amyloid hypothesis, the tau hypothesis assigns cause not to beta-amyloid plaques, but to neurofibrillary tangles (NFT) caused by the tau protein (Mohandas, Rajmohan, & Raghunath, 2009). The tau protein functions to stabilize microtubules in neurons, but in AD, tau proteins are hyperphosphorylated and cause the microtubules to depolymerize, which triggers tau proteins to misfold (Mohandas et al., 2009). This in turn creates a chain reaction, which can be spread from cell to cell, as other tau proteins are triggered to misfold the same way, leading to tau oligomers aggregation (TauRx Pharmaceuticals, n.d.). Afterwards, the tau oligomers develop into paired helical filaments and form NFT which accumulate inside neurons, hindering normal cell processes (Mohandas et al., 2009). This whole process is simplified and shown in Figure 2. Eventually, neurons...
filled with NFT burst and cause neurodegeneration (TauRx Pharmaceuticals, n.d.).

Figure 2. Schematic illustration of the tau hypothesis. Shows hyperphosphorylation and aggregation of tau to form neurofibrillary tangles

The cholinergic hypothesis describes a diminution of acetylcholine (ACh) at synapses in the brain, otherwise known as a cholinergic deficit, as a major cause of AD (Ferreira-Vieira, Guimaraes, Silva, & Ribeiro, 2016). Acetylcholine, a common neurotransmitter, plays a significant role in thought processing, attention, and short-term memory (Ferreira-Vieira et al., 2016). Healthy neuronal synapses store acetylcholine in vesicles in the presynaptic neuron and release it into the synapse when an action potential arrives at the presynaptic axonal terminals as illustrated in Figure 3 (Ferreira-Vieira et al., 2016). ACh then binds to the receptors of the postsynaptic neuron to relay the electrochemical signal. To ensure a transient response, acetylcholine is rapidly cleared from the synaptic cleft by (i) diffusion, (ii) breakdown into choline and acetate by acetylcholinesterase, and (iii) reabsorption into the presynaptic neuron (Ferreira-Vieira et al., 2016). In patients with Alzheimer’s, a progressive death of cholinergic neurons results in an insufficient amount of acetylcholine, impeding synaptic activity and signal transduction, and causing learning impairment and memory loss (Ferreira-Vieira et al., 2016).
1.4 Current Therapeutic Strategies for AD

Therapeutic strategies for clearance or reduction of beta-amyloid have not succeeded in producing clinical benefits. Current effective drugs for Alzheimer’s mainly target the cholinergic system, preventing acetylcholine from getting broken down into acetate and choline, therefore maintaining a sufficient amount of acetylcholine at synapses (Ferreira-Vieira et al., 2016). Agents like Donepezil, Rivastigmine, and Galantamine, ease symptoms of AD but do not cure the disease completely.

These beneficial drugs target one of the brain’s neuromodulatory systems that uses acetylcholine and plays a significant role in cognitive processing and short-term memory (BrainFacts.org, 2012). The drugs that target cholinergic neurotransmission maintain a healthy level of ACh despite a gradual decrease in the number of cholinergic neurons associated with AD.

The treatment idea this paper outlines builds on the success of these drugs but uses a different approach. Instead of maintaining reasonably high ACh levels, this program aims to replenish the population of cholinergic neurons using stem cell technology by generating new cholinergic neurons from induced pluripotent stem cells (iPSCs).

1.5 Stem Cell Technologies and a New Approach

This paper outlines a novel treatment approach that relies upon the potential of stem cell technologies. Stem cells are cells which can either replicate to form new stem cells or differentiate into other types of cells such as muscle cells, nerve cells, bone cells, or skin cells (Mayo Clinic, 2022). They are the only type of cells that have the ability to become any specialized cell in the body. Stem cells are of interest to scientists as they can be used to differentiate into any desired cell type to replace damaged cells caused by diseases (Mayo Clinic, 2022).

In 2006, Japanese scientist Shinya Yamanaka made a revolutionary breakthrough in the field of stem cell technology and became a Nobel Prize laureate in 2012 for his work (Rogers, 2020). Yamanaka’s lab discovered that stem cells could be developed from somatic cells using four specific transcription factors – Myc, Oct3/4, Sox2, and Klf4 – which reversed specialized cells back to a pluripotent stem cell state (Rogers, 2020). Prior to this discovery, stem cells could only be derived from embryos and caused controversy surrounding ethical issues of destroying a human embryo, which were also linked to debates over abortion rights, as well as unpredictable immunity reactions in test subjects. Yamanaka’s work on creating induced pluripotent stem cells provided a
better solution and a safer alternative.

The overall procedure for the treatment development program outlined by this paper is founded on a study by neuroscientist Kwang-Soo Kim and neurosurgeon Jeffrey S. Schweitzer titled “Personalized iPSC-Derived Dopamine Progenitor Cells for Parkinson’s Disease” (Schweitzer et al., 2020). Individuals with Parkinson’s disease suffer a shortage of the neurotransmitter dopamine, which restrains muscle control and movement, causing muscle spasms, loss of balance, and other symptoms (Begley, 2020). Kim had been working on successfully differentiating induced pluripotent stem cells into dopamine neurons free of mutations, and by 2016, his experiments proved effective in mouse trials as their Parkinson’s symptoms were improved after treatment of the injected neurons (Begley, 2020). Schweitzer and his team harvested skin cells from a 69-year-old patient with Parkinson’s disease and reverted them into iPSCs before differentiating them into dopamine progenitor cells (Schweitzer et al., 2020). In September of 2017, Schweitzer transplanted the personalized dopaminergic cells into the patient through MRI-guided surgical procedures (Begley, 2020). The treatment proposed by this paper would extend this therapeutic approach to target AD by focusing on replenishing cholinergic neurons instead of dopaminergic neurons as a replacement therapy.

Although the idea of injecting foreign cells into the brain may appear risky and futuristic to many, this methodology is involved in many clinical trials regarding neuroscience. One example is the study mentioned above and another example is a current study being conducted by researchers at the University of California San Diego School of Medicine. The team, lead by Mark Tuszynski, is injecting brain-derived neurotrophic factors (BDNF), proteins needed in promoting neuron growth and regeneration, into the brains of 12 patients with AD or mild cognitive impairment (UC San Diego Health, 2021). Their experiment differs from this proposed treatment program in that they are injecting viral vectors carrying BDNF into the brain to deliver the proteins into existing neurons, a technique known as gene therapy (UC San Diego Health, 2021).

Despite differences in details, the common denominator between these studies and my novel treatment approach is the delivery of foreign genetic material into the brain as a form of treatment, whether the materials being injected are dopaminergic neurons, modified viruses carrying proteins, or cholinergic neurons differentiated from iPSCs.

Although there are risks involved with this form of therapeutic approach, they are outweighed by the potential clinical benefits.

2. Methods

Below are methods that I envision would be employed for the development of this treatment program.

2.1 iPSC Development and ACh Cell Characterization

All somatic cells used for differentiation would be harvested from patients who have provided their full consent for the procedure and who possess a full knowledge of how these biopsies will be used. First, a skin biopsy would extract skin fibroblasts from patients to be converted into iPSCs using specific transcription factors. Next, researchers would screen the iPSC line with whole-exome sequencing, isolating a single cell to be further characterized and cloned into basal forebrain acetylcholinergic progenitor cells (APCs). Multiple tests would ensure that the APCs remain free of cancer-associated and neurodegeneration-associated mutations, that they secrete ACh, and whole-cell patch-clamp recordings can be used to test that the ACh cells exhibit electrophysiological properties of cholinergic neurons. Injecting undifferentiated stem cells into the brain can cause uncontrolled cell proliferation or unregulated cell replication. To mitigate this risk, treatment of Quercetin would be needed to eliminate undifferentiated iPSCs and after sufficient time, researchers would examine cell cultures to verify the absence of undifferentiated iPSCs.

2.2 Pre-Clinical Mouse Study

Researchers would use established mouse models exhibiting characteristics and symptoms of AD (APP_SweDI; overexpressing amyloid precursor protein (APP) with the Swedish K670N/M671L, Dutch E693Q, and Iowa D694N mutations) for pre-clinical mouse experiments (Foidl, Do-Dinh, Hutter-Schmid, Bliem, & Humpel, 2016). APCs developed in Stage 1 would be injected into the basal forebrain of the APP mice. After a couple of months of cell development, researchers would perform a series of behavioral tests focusing on spatial and working memory on the APP mice, such as the Morris water maze, the radial arm maze, the Y-maze, and the T-maze. Subsequently, researchers would conduct histological brain examinations on the mice brain grafts and stain the samples with human markers to check for the survival and integration of transplanted human
iPSC-derived neurons. The development team would analyze tests results and if the team identifies improvement, they would be able to proceed with clinical trials in Stage 3.

2.3 Clinical Trials

The team would need to recruit two groups of volunteers. The first group would be patients who have been diagnosed with early or middle stage of AD, exhibiting symptoms such as mild to moderate cognitive impairment and memory loss, as this treatment program is intended to reverse symptoms in the earlier stages of the disease. The second group would be a control group with volunteers who do not have AD and would not receive treatment.

Recruited patients would be provided with a detailed overview of the plan and procedure of the clinical trials, and further progress would only be made with their full consent. Next, stem cell biologists and lab scientists would harvest the cells of patients from group 1 using a skin biopsy to generate the iPSCs. Following cell development and quality control outlined in 2.1 ACh Cell Development, surgeons would transplant APCs into each patient’s basal forebrain using an MRI-guided stereotactic surgical procedure. Neurologists and research technicians would consult computed tomographic (CT) scans on patients to validate accurate placement of the injected cells within the basal forebrain. Researchers would use magnetic resonance imaging (MRI) scans to identify signs of tumors or hemorrhage that would threaten the health and safety of patients. The team would record results of behavior monitoring every three months prior to the first year and every six months for each subsequent year. Researchers would use positron-emission tomography (PET)-CT scans throughout the trial to visualize presynaptic acetylcholine activity and record any instances the treatment did not perform as expected, supported by recounts from the patients. To evaluate the behavioral performance of patients, the research team could use the Sandoz Clinical Assessment-Geriatric (SCAG) scale, the Integrated Alzheimer's Disease Rating Scale (iADRS), and the Alzheimer’s Disease Assessment Scale–Cognitive Subscale (ADAS-Cog). Furthermore, results would be analyzed by comparing brain imaging before the treatment to after the treatment as well as comparing with that of the control group.

2.4 Scientific Team

This treatment development program would require a team of stem cell biologists, lab scientists, neurologists, surgeons, experts in pharmacology, medical doctors, and research technicians. Stem cell biologists and lab scientists would be vital in the process of developing the iPSCs and manipulating the cells for desired results. Neurologists and surgeons would be key in implanting the neurons into animal and test subjects, as well as conducting functional MRI scans and behavioral testing. Medical doctors would primarily assist with clinical trials and care of patients, and research technicians would support the research process in general. A director would supervise the entire process along with the rest of the team.

3. Results

Overall, this treatment plan would restore normal levels of ACh in the brain of AD patients by injecting new nerve cells developed from patient-derived iPSCs. Patients would not need any immunosuppressive drugs for surgery as injected cells are developed from their own skin fibroblasts. The approach to carrying out this treatment plan would follow three steps: stage 1, ACh cell development; stage 2, pre-clinical experiments in mice; and stage 3, human clinical trials, as outlined in Figure 4. Stage 1 and stage 2 should each take approximately 1 year, and stage 3 should take around 1 to 2 years with the whole treatment program spanning 3 to 4 years. It is important to clarify that stages 1 and 2 are essentially lab work acting as the prerequisite for stage 3, where patients will receive treatment in clinical trials.
With regards to the pre-clinical mouse experiments, previous research suggests that scientists can expect to see improvement in AD induced behavior in APP mice as soon as 12 weeks after transplantation (Song, B., et al., 2019). Although the time it takes to see results vary for each behavioral test, depending on difficulty, researchers should find significant improvement between 16 to 20 weeks after transplantation for simpler tasks, and around 24 weeks for more complicated tasks, such as the Morris Water Maze. (Song, B., et al., 2019). The resulting improvements from the transplanted cholinergic neurons should be well sustained, as long as up to 52 weeks (Song, B., et al., 2019).

For clinical trials, two surgical injections of ACh neurons delivered bilaterally, which can be delivered in one surgical procedure or alternatively in two, should be sufficient for each AD patient. Researchers should expect an overall improvement of cognitive behavior and function through using the rating scales listed in 2.3 Clinical Trials by comparing results measured before the implantation and at regular intervals after the implantation.

4. Discussion

If successful, this treatment protocol will impact the medical, economic, and social facets of Alzheimer’s disease treatment and patient experiences.

4.1 Medical Impact

This method of iPSC-developed neurons can be used for other fields in medicine, beyond neuroscience, such as for cancer treatment, diabetes, heart disease, and more. Although iPSC technology in the treatment of diseases is still a novel concept, as research involving stem cells expands, the potential of stem cell technology may demonstrate a wide array of benefits. This experimental treatment program, if successful, will showcase advantages of iPSCs for personalized treatment, and demonstrate its potential in various aspects of medical treatment in the future.

4.2 Economic Impact

If successful, this protocol will reduce the cost of medication for AD. After AD patients undergo nerve cell injections, their need for medication will decrease over time as new neurons supersede the effects of AD drugs. Currently, the total cost for treatment of all AD patients in America is around $305 billion, making AD one of the costliest diseases to treat (Wong, 2020). With new drugs being developed, costs for treatment can climb to $56,000 per year for one individual. However, with pharmaceutical demand decreased by effective stem cell treatments such as this, drug expenditure needs for AD victims may decrease drastically.
4.3 Social Impact
This proposed treatment would free AD patients from requiring prescribed medication daily, removing from their daily routines the reminders of the burdens of their disease and restoring a sense of living normally. This retained sense of normalcy will affect these patients’ social activities. With improved coordination of motor movement, patients will no longer feel the need for assistance with basic activities like walking and eating, restoring independence to AD patients. The treatment will also subdue erratic behavior caused by memory loss, relieving one of the more painful impacts of AD felt by patients, their families, and others within their social circles.

Competing Interests Statement
The authors declare that there are no competing or potential conflicts of interest.

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