

**CAN STEM CELLS CURE OUR MINDS?
AN INVESTIGATION INTO USING STEM CELLS TO TREAT
ALZHEIMER'S DISEASE**

BY

JULIET BOTTLE

PASS WITH DISTINCTION

**RESEARCH PAPER
BASED ON
PATHOLOGY LECTURES
AT MEDLINK 2008 AND VET-MEDLINK 2008**

Abstract

A study conducted by the London School of Economics showed that by 2025, 1 in 3 people will be affected by Alzheimer's Disease (AD), as either a sufferer, carer or relative. This paper is a logical investigation into the use of stem cells to cure dementia, in particular AD. The treatment focuses on the introduction of neural stem cells to the brain, which would differentiate and mature into new neurons. In this paper, ideas will be proposed concerning how to procure suitable stem cells which will differentiate and can be used for tissue repair. The use of chemical signalling in differentiation will be investigated, and how to introduce stem cells into the brain in a safe and effective way. The uses and problems of the proposed methods, and the ethical issues surrounding the use of embryonic stem cell therapies will be assessed.

Introduction

There is an immediate need for effective treatment for AD. The LSE and Institute of Psychiatry have warned that the NHS faces a dementia crisis, and delaying the onset of dementia is estimated to reduce deaths in the UK by 30,000 a year. Currently, dementia costs the NHS £17 billion a year, rising to about £50 billion in the next 20 years. AD accounts for 55% of dementia in the UK (Knapp, 2007).

Structure of the Brain

The brain is divided into various areas (Figure 1). The folded cerebral cortex is the upper/outer layer of the brain, and the cells within are roughly divided into two groups: neurons and glia. Neurons are responsible for relaying of information within the brain. Gaps between neurons are called synapses and chemical transmitters conduct signals across these gaps. Glial cells contribute to the maintenance and repair of the neurons. Oligodendrocytes (glial) produce the myelin sheath which surrounds and insulates the axon of a neuron, improving its efficiency at relaying messages. Astrocytes maintain the physical structure of the brain, have a role in synaptic transmitter uptake and release, and in repair of damaged neurons.

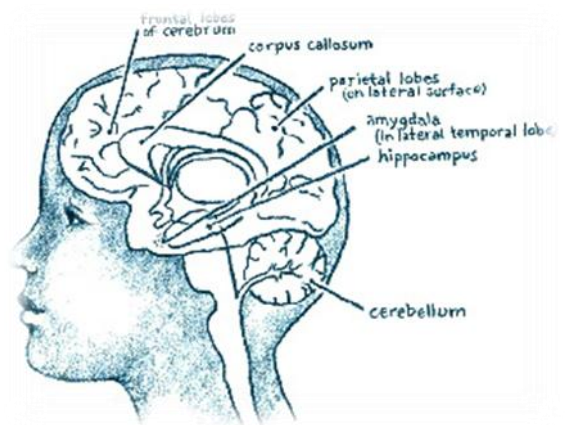


Figure 1. Diagram of the main areas of the brain

Pathology of Alzheimer's

AD is caused by the damage and loss of neurons in the cerebral cortex, including the hippocampus and frontal lobe (Figure 1). These areas of the brain are involved in cognitive processes. The frontal lobe is believed to be involved in processing memory, emotion and language. The damage is caused by a process in which an enzyme acts upon APP (amyloid precursor protein) which is a protein penetrating through cell surface membranes of neurons. The enzyme breaks down the protein and the resulting fragments of β -amyloid form clumps, called senile plaques, outside neurons (Figure 2).

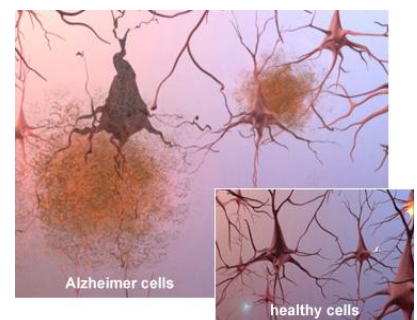
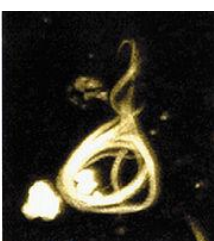


Figure 2, comparison of the neuron forest of a healthy person with that of a person with advanced Alzheimer's, showing the plaques and tangles

Figure 3, Microscopy image of a neurofibrillary tangle, conformed by hyperphosphorylated tau protein



(Yankner, 1989) The disease's neuropathology involves a protein known as tau protein. The protein which normally stabilizes microtubules (spindle fibres) in cells undergoes a chemical change, and pairs with other threads forming fibrous tangles within the cells. (Figure 2&3) The combination of the tau

tangles and β -amyloid plaques contribute to the disintegration of neurons and synapses. A more recent study has shown that β -amyloid plaque may be cytotoxic to glial cells, particularly oligodendrocytes, and that the disintegration of these cells may be a cause of the further apoptosis (cell death) of neurons, since these cells produce the myelin sheath that insulates neurons. (Roth, 2005).

Stem Cells

“Stem Cell” is an umbrella term; there are different types and classes, with varying potentiality (potential to differentiate into different cells). Embryonic Stem Cells (ESCs) are the first cells of human development. They are undifferentiated and hence unique to other cells because they can potentially become any sort of tissue – they are totipotent. ESCs in the embryo arrange themselves into 3 distinct germ layers which give rise to all the tissues in the body. Each germ layer contains distinct pluripotent ESCs. For example ESCs from the ectoderm, the external germ layer, may differentiate into tissues of the epidermis and associated structures, as well as the central nervous system (CNS) and brain. These ESCs are self-renewing – they will divide endlessly, and are described as having immortality.

Neural Stem Cells (NSCs) in development are pluripotent. They can only differentiate into a few types of cell: neuronal precursor cells which then differentiate into neurons, or glial precursor cells which differentiate into a type of glial cell. This could be, for example, an astrocyte or an oligodendrocyte.

Differentiation occurs when a cell divides by mitosis. The determination of cell fate occurs either through conditional or autonomous specification. Conditional specification involves extrinsic molecular chemicals acting as signals for a fate. The chemical signal could be either inhibitory or inducive of a particular cell fate. Autonomous specification, in contrast, occurs because of differences in the molecules in the cytoplasm that each daughter cell inherits from the parent cell during cytokinesis. This type of differentiation is unaffected by chemical signals, and thus the type of differentiation of stem cells is very important. A stem cell which differentiates by conditional specification will have its cell lineage subjected to its local surrounding, and chemical signalling. Recent developments have shown that chemical signalling is involved in the differentiation of neurons (Breunig, 2008) and glial cells (Kessariss, 2008). A key signalling device is known to be the Sonic Hedgehog Homologue: a protein whose concentration can affect the fate of stem cells. (Nusslein-Volhard, 1980).

The focus of this paper will address the question: How can stem cells be used to repair damage to neurons which are damaged by the β -amyloid plaques and neurofibrillary tangles in the brain? ESCs are self-renewing and potent; they can be multiplied in vitro, and then injected into the body to differentiate into new cells to replace the damaged neurons or glia.

The possibilities of using adult NSCs, already latent in the body, to differentiate into the neurons are addressed. If these cells could be extracted, or alternatively be persuaded to activate and multiply within the body, they could potentially be used to repair and replace damaged neurons.

Discussion

Embryonic Stem Cells

ESCs provide an excellent stem cell which can be used to create neural or glial precursor cells. They have displayed potency to differentiate into any cell, either in vitro or in vivo. In this proposed treatment of AD, the therapeutic cloning or nuclear transplantation of the sufferers DNA from a diploid cell into a host gamete (which has had its own DNA removed) would produce a genetically identical blastocyte consisting of undifferentiated ESCs. Embryonic stem cells are known to begin differentiation after eight cells are present in the blastocyst so before this stage, one ESC would need to be extracted and cultured in an environment

that would signal it first to multiply, and then to differentiate into neurons. The ways to differentiate the ESCs into neurons remain a challenge and research is required into the technique of producing large numbers of workable NSCs, which will differentiate into functioning neurons. However, this is not “Star Trek Science”. Previously NSCs have been obtained from hESCs by co-culturing them with stromal cells (which are found in connective tissue). This is a good way of producing quantities of NSCs from ESC’s. However, recently a study provided more detailed insight into the signalling which would allow these ESCs to follow NSC lineages. It showed that inhibiting a growth factor called Bone Morphogenic Protein, by using the inhibitor Noggin protein, encourages not only neural cell differentiation of hESCs but also encouraged the NSCs to follow neuronal lineages (Gerrard, 2005). So ESCs seem to show great promise in terms of their clinical application into curing AD and are strong candidates for producing neurons. The strides made in recent research are shown by the success of Yi-Sun’s team in deriving NSCs which had a neuronal differentiation of 70%– 80%, and that all the neurons had a functional synaptic network, meaning they could relay messages (Sun, 2007). (Figure 3)

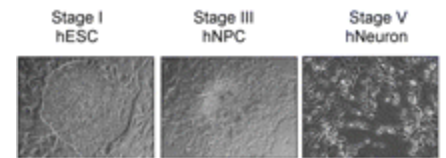


Figure 3 - stages of Sun's experiment. Left, human embryonic stem cell. Middle, human neural precursor cell. Right, Human Neurons

However, the use of hESCs in treating AD would be extremely controversial. Firstly, the concept of human cloning is opposed by some scientists who are opposed to nuclear transplantation. However it can be argued that the blastocyst produced would be destroyed after a few days, specifically, by the nature of the treatment, before any of the cells have begun to differentiate. This means that the ball of cells is not the beginnings of any tissues or organs. In this sense a cloned human would never be brought to life. However, the definition of the beginning of life is a grey area, and although currently research is allowed on cloned embryos, cloning for treatment is banned in the UK. A political and social debate is needed to address these barriers to the success of the treatment.

Additionally, pro-lifers would be against the destruction of such an embryo and would argue that producing a human embryo and then destroying it constitutes murder. However, as far as research goes, there are thousands of spare embryos frozen as a result of IVF treatment. Research conducted in the USA revealed that 60% of couples would be happy to donate their spare embryos for research purposes. However, more difficult is persuading opponents and religious groups that human cloning and then destruction of the clone would be moral. There is bound to be much opposition to using human embryos for research or clinical purposes, and this should be taken into consideration when weighing up the options as to which cell is the best candidate for treating AD

Adult Neural Stem Cells

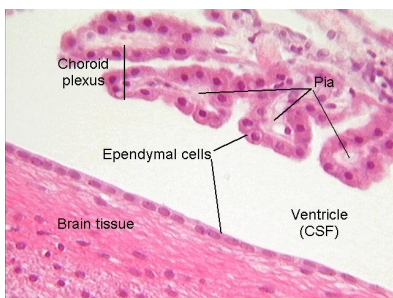


Figure 4: Light microscopy slide showing human ependymal cells lining a ventricle. Stained with H&E.

An alternative stem cell to use in the treatment would be NSCs harvested from the sufferer himself. Adult stem cells or somatic stem cells are multipotent tissue stem cells which have are self-renewing and multipotent. Previously, NSCs were not believed to exist and many scientists believed that the human nervous system was fixed from birth and not able create new cells or neurons. However, NSCs have recently been proved to exist in the human brain. (Eriksson, 1998). Contention still remains as to where exactly the stem cells are, although they are believed to lie with the ependymal cells which line the ventricles in the brain (Figure 4). The ventricles of the brain contain Cerebral Spinal Fluid, (CSF), which consists mostly of water and a low concentration of plasma proteins.

Logically, if these stem cells could be activated to divide, they may enter the CSF, and could then be extracted through a relatively simple procedure, the lumbar puncture, from the spinal cord. This would mean

no invasive biopsy would be needed to extract the cells from the brain, reducing risk of brain injury. Similarly, the drug used to stimulate these cells to divide could also theoretically be administered in the CSF. The only current problem is that scientists do not know the exact way to activate the stem cells to divide. A recent study however, suggested that mood stabilizing drugs already on the market may do exactly this, and help to activate the stem cells to divide, by way of activating a signalling pathway known as Notch. (Higashi, 2008). The authors of the study suggest this be used as a way to regenerate the nervous system following injury, but this could also be used to increase numbers of stem cells in the CSF, so they can be extracted for use in Alzheimer's treatment. However, more studies are needed to make sure these stem cells are fully pluripotent and self-renewing. Once the cells have been extracted, they can be inserted into the brain into the exact area which has been damaged by AD

There are however possible problems to extracting stem cells from the CNS in this way. The treatment of increasing the number of stem cells in the spinal fluid by activating the ependymal cells could be dangerous because if too many stem cells are activated in the brain it could potentially cause a brain tumour. Although previously brain tumours were thought to originate sporadically from glial cells, a recent study on mice has shown that they originate from the NSCs lining the ventricles. (Llaguno, 2009).

To overcome this problem, it would be sensible to administer regular brain scans after the extraction of the NSCs to scan for brain tumours.

Chemical Signalling

It is known that cells can differentiate in 2 broad ways. Either they differentiate autonomically (automatically) or conditionally. On the other hand, differentiation can be affected by conditions. These conditions may be caused by chemical molecules in the environment, for example a soluble substance or growth factor.

Conditional specification of cell fate must therefore provide scope for scientists to "nudge" stem cells to differentiate into a specific cell. If a chemical is shown to increase the differentiation of neurons, it could be added to a stem cell culture to increase the chance of neuronal differentiation. Similarly, if a chemical is known to inhibit a factor which might *prohibit* neuronal differentiation, this chemical could be added to the culture to increase neuronal differentiation.

It has been shown in a recent study neuronal differentiation occurred in mice when targeted apoptosis, as a result of photolysis, had occurred in neurons. The stem cells injected into this area had an increased rate of neuronal differentiation. (Snyder, 1997) This suggests that some the neuronal differentiation can be affected by conditions in the local area, specifically by chemical signals arising from apoptosis. If it were possible to mimic the chemical environment produced when neurons undergo apoptosis it should increase the chance that stem cells will differentiate into the useful neurons. There is scope here to "fine tune" NSCs into having the optimal differentiation ratio of neurons to glia. Furthermore, as discussed below, this characteristic of stem cells may give us an advantage, since NSCs do undergo apoptosis, so an ideal environment for neuronal differentiation may already be present in AD sufferer's brains.

Is nudging the neural stem cells necessary?

There has been recent research into the use of chemical signals to "tweak" the differentiation of stem cells into different types of neurons, or glia. However, in terms of the application of the stem cells clinically, this may be less necessary than thought. A study showed that in rats, NSCs were able to differentiate and integrate themselves into functioning neurons, possibly using cell to cell interactions rather than a dissolved chemical (Englund, 2002). This could also apply in humans, suggesting that if NSCs are injected then cell to cell interactions in the local tissue environment can help to "guide" the stem cells to differentiate into functioning and integrated neurons. However, the authors remarked that because the interactions are intercellular, transplanting stem cells into an area of the brain where all the neurons are already destroyed may not work, and the stem cells would not differentiate. This indicates that in terms of clinical application, the earlier the treatment, the more effective it would be, since there would be more neurons in the environment to "guide" the stem cells. Treating too late into the disease progression would mean fewer stem

cells would differentiate into neurons, therefore less effective tissue repair. This in turn underlines the importance of AD awareness in the public, and the use of PET scanning to identify the disease early on.

Inserting the stem cells

Before the stem cells can be inserted, the areas damaged by the AD should be identified. Since AD affects everyone differently, these will be specific for the individual. To identify the exact location of damage, a marker which attaches to the β -amyloid protein plaques could be released into the blood stream, and this marker would then be detectable by a brain scanner, for example a Positron Emission Spectrum (PET) Scan. This idea has been realised for the first time when a compound called FDDNP was used in conjunction with PET scanning. FDDNP binds to tau and to β -amyloid. This means that not only does it show areas where neuronal degeneration has already occurred to an extent where it is recognisable using a scan which looks for neuron loss, but also highlights parts of the brain where neuron loss is just beginning, but there is already a build up of tau and senile plaques, and could benefit from stem cell injections (Figure 5).

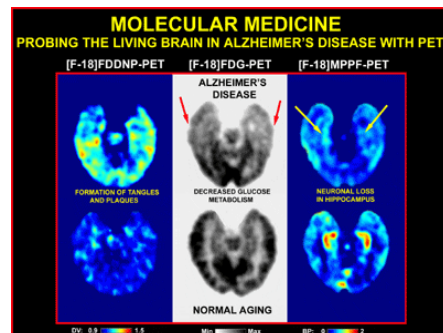
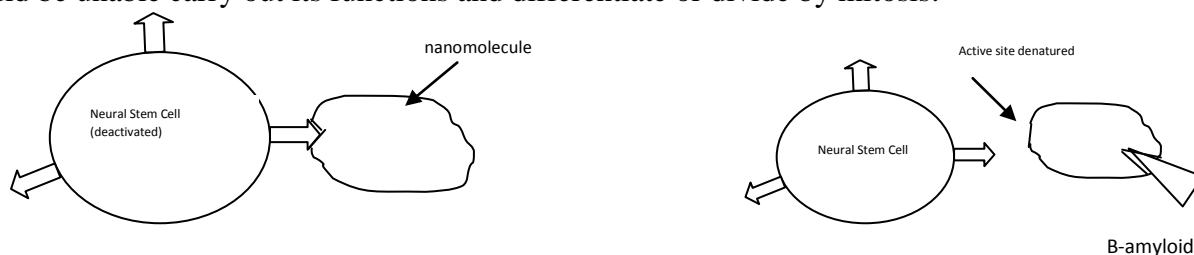


Figure 5: PET Scans of an AD brain and a normal aging brain. Left: areas of Tau or senile plaques highlighted, Centre: decreased glucose metabolism, Right, neuron loss

A nanomolecular development?

Injecting the stem cells is the next logical step in the proposed treatment of AD. Whether derived from embryos or from human NSCs, in order to achieve effectiveness they could be inserted directly into the brain in the places which are damaged by senile plaques or tau protein fibrils, using an ultra-fine needle. However, this technique poses dangers, like any invasive neurosurgery, for example haemorrhaging of blood vessels in the brain. Additionally, it might not lead necessarily to the stem cells activating in exactly the right place in the brain. They may, for example, diverge into the blood stream in the tiny capillaries which run through the brain, and be carried away to another area. The presence of active stem cells in the brain which are then transported to unknown areas could also pose a risk of cancer developing, since the stem cells will be dividing continuously, which may result in a tumour.

The new idea expounded in this paper would be to contain the stem cells in an active form until they reach the sites of neuronal degenerations, (or more specifically, of β -amyloid plaques surrounding the dying neurons). Here they need to be activated so that they could divide and differentiate into neurons which would mature and replace the dying ones. My proposal is to attach a specifically designed nanomolecule onto a membrane protein, or glycoprotein, in the plasma surface membrane of the stem cell. To avoid the difficulties of having to attach molecules individually onto each cell, this would be achieved by designing the nanomolecule to have a receptor site complementary to the molecular shape of the protein, or the carbohydrate chain of the glycoprotein. If a culture of stem cells and these nanomolecules were mixed, the nanomolecules should attach themselves to the stem cells by collision. There could easily be more than one of these nanomolecules attached to the stem cell. The stem cell would now theoretically be inactivated, since it would be unable carry out its functions and differentiate or divide by mitosis.



Nanomolecule binds to NSC's protein
NSC is inactive

Nanomolecule binds to β -amyloid fragment, releasing and activating stem cell. NSC is active

Figure 6 - A diagram of a theoretical nanomolecular "vector" which could be used to safely deliver NSCs to Alzheimer's damaged locations

The inactive form of this molecule would be injected into the patient's blood stream into an artery going towards the brain, for example the carotid artery. The other pole of the nanomolecule would have a receptor site complimentary to the shape of the β -amyloid fragments in the senile plaques. When the molecule came into contact with the β -amyloid, it would bond with the fragment. The nanomolecule should be designed such that its shape and/or charges change sufficiently when it bonds with the β -amyloid fragment at one end (mimicking the induced fit principle), that the stem cells is released at the other end, and is activated to divide at exactly the site where repair and re-growth of tissue is needed.

Such a molecule would be within the scope of nanomolecular science, and is an example of how two new phenomena, nanomolecules and stem cells, can be combined to provide an effective treatment against AD

Conclusion

As a summary, my investigation has demonstrated to me that using cloned embryonic stem cells to treat AD may be a better choice in terms of their potency to develop into neurons. However, due to ethical issues, adult NSCs could be a useful alternative since they are much less controversial. I have discovered that using chemical signalling to nudge NSCs to differentiate may be extremely useful, but that further research is needed on this point. However, I have also deduced that the brain suffering from AD may actually provide a good environment in which the stem cells can work effectively. I have postulated an original idea of how to use a nanomolecule to safely deliver stem cells to exactly the right places in the brain, as well as suggesting how the whole treatment may be used in clinical practise, using frequent PET scans to identify problem areas and treat them accordingly.

This paper has been an investigation into a possible method of treating Alzheimer's disease. It is intended to show that stem cell research has never been more necessary. Clearly there is a vast amount of work still to be done, but the investigation has also thrown light on discoveries that should give every sufferer hope. The theories and methods postulated in this paper are within reach of current science. The main barriers to the idea's becoming reality, however, will be factors such as "moral" opposition to research on embryos and human cloning, and the use of these methods in treatment. I have suggested ideas such as using adult NSCs to provide treatment. However, research must be carried out using embryonic stem cells, because these may eventually prove to be far more effective than using adult NSCs. This paper can be used as evidence for the case of allowing scientists freedom to carry out research on embryonic stem cells because it demonstrates huge benefits that the exciting field of stem cell treatment can bring to millions of people.

Bibliography

WORKS CITED

- Breunig, S. A. (2008). Primary cilia regulate hippocampal neurogenesis by mediating sonic hedgehog signalling. *Proceedings of the National Academy of Sciences* , 13127-13132 .
- Englund, B. W. (2002). Grafted neural stem cells develop into functional pyramidal neurons and integrate into host cortical circuitry. *Proceedings of the National Academy of Sciences* , 17089-17094.
- Eriksson, P.-E. A. (1998). Neurogenesis in the adult human hippocampus. *Nature* , 1313 - 1317.
- Gerrard, R. C. (2005). Differentiation of Human Embryonic Stem Cells to Neural Lineages in Adherent Culture by Blocking Bone Morphogenetic Protein Signaling. *Stem Cells Vol. 23* , 1234 -1241.
- Higashi, M. B. (2008). Mood Stabilizing Drugs Expand the Neural Stem Cell Pool in the Adult Brain Through Activation of Notch Signaling. *Stem Cells* , 1758 -1767.
- Kessariss, P. R. (2008). Specification of CNS glia from neural stem cells in the embryonic neuroepithelium. *Philosophical Transactions of the Royal Society B: Biological Sciences* , 71–85.
- Knapp, C.-H. W. (2007). Cognitive Impairment in Older People: future demand for long-term care services and the associated costs. *International Journal of Geriatric Psychiatry* .
- Llaguno, C. K. (2009). Malignant Astrocytomas Originate from Neural Stem/Progenitor Cells in a Somatic Tumor Suppressor Mouse Model. *Cancer Cell* , 45-56.
- Nusslein-Volhard, W. (1980). Mutations affecting segment number and polarity in Drosophila. *Nature* .
- Roth, R. A. (2005). Oligodendrocytes damage in Alzheimer's disease: Beta amyloid toxicity and inflammation. *Biological Research v.38* , 381-387.
- Sun, W. X. (2007). Integrative genomic and functional analyses reveal neuronal subtype differentiation bias in human embryonic stem cell lines. *Proceedings of the National Academy of Sciences* , 13821-13826.
- Yankner, D. F.-K.-G. (1989). Neurotoxicity of a fragment of the amyloid precursor associated with Alzheimer's disease. *Science* , 417-420 .

WEBSITES USED

- http://www.timesonline.co.uk/tol/life_and_style/health/article5654911.ece.
- http://www.today.uci.edu/news/release_detail.asp?key=1801.
- http://www.timesonline.co.uk/tol/life_and_style/health/article5654911.ece
- <http://www.parliament.uk/documents/upload/postpn278.pdf>
- <http://alzheimers.about.com/library/blfrontal.htm>
- <http://interactive.snm.org/index.cfm?PageID=2601&RPID=627>
- <http://emedicine.medscape.com/article/1134817-overview>
- <http://en.wikipedia.org/wiki/Alzheimer%27s>
- http://neuromedia.neurobio.ucla.edu/campbell/nervous/wp_images/199_brain_ependyma.gif