The future treatment of Alzheimer’s disease with brain derived neurotrophic factor

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ABSTRACT

Alzheimer’s disease (AD) is the most common form of dementia in people aged 65 and older, accounting for up to 80% of dementia cases. It is a progressive degenerative disease of the brain with no official cure found so far. During the course of the disease, neurons (brain cells) slowly die and synapses (connections between them) wither, thereby impairing a person’s cognitive abilities such as attention, memory, learning and abstract thinking. Consequently, gradual neurodegeneration leads to the shrinking of the person’s brain and subsequently to their death. One possible future treatment would involve the use of BDNF – protein, synthesised in our brains, which has been known from its neuroprotective abilities, that could be administered to the brain in many ways including exercise, gene therapy, stem cells and drugs.

INTRODUCTION

At the beginning of the 20th century, Alois Alzheimer a German neurologist described ‘strange, severe, progressive disease of the cerebral cortex with irregular atrophy of the brain’ (Fig.1). As life expectancy rose rapidly in those times, partly because of industrialisation, the incidence of disease has been increasing every consecutive decade until now, when it has reached its apogee.

In the early stages of AD, symptoms such as amnesia (loss of short term memory - STM) are vague as they resemble the normal signs of ageing. However, the disease worsens as it progresses. The next stages include aphasia, apraxia and agnosia – impairments of language, movements and recognition skills respectively. The AD brain is considerably shrunken when compared to a normal healthy brain (Fig.2 – AD brain at left). There are many hypotheses trying to explain this neuropathology. The most famous are cholinergic (AD begins as deficiency in the production of a vital neurotransmitter – acetylcholine – Fig.3), tau (deposition of over-phosphorylated microtubule associated tau protein = neurofibrillary tangles that disrupt nerve impulses) and amyloid (accumulation of neurotoxic amyloid plaques between neurons in the brain = senile plaques). As there is no proper medication, the main aim of the treatment is to alleviate the symptoms and to delay the progression of the disease.

‘Mens sana in corpore sano’ – ‘A sound mind in a healthy body’ said pre-Socratic Greek philosopher Thales. Although this statement was written in ancient times, it certainly hasn’t been devalued, especially nowadays, when the intriguing possible connection between body and mind is being put forward and examined again, a division between psychology and medicine having been the traditional convention for many years. Living a healthy lifestyle and exercising has been propagated almost everywhere from TV fitness programs and radio auditions through the Olympics/Paralympics and compulsory physical education in schools.
Being fit, healthy and young is purportedly the main motive of the 21st century. Exercise has proved repeatedly to be essential and beneficial in the adequate functioning of our body, decreasing risk factors for heart disease such as blood cholesterol levels, hypertension, diabetes and even cancer, stimulating angiogenesis (growth of new blood vessels), increasing life expectancy, lowering levels of depression and anxiety and overall improving the quality of life.
Furthermore, researchers at Columbia University have provided the first evidence of a structured exercise training program being linked with the augmentation in neurogenesis in the region of the hippocampus, an area particularly prone to AD neuropathology, called dentate gyrus. They found a positive correlation between neuronal proliferation and changes in cerebral blood volume in mice that had been running voluntarily for two weeks on a treadmill, using a magnetic resonance imager (MRI). Subsequently, they conducted MRI on the small group of middle-aged people, who had been systematically exercising for three months. Researchers investigated exercise-induced changes in the participants' cerebral blood volume in the dentate gyrus area and deduced the extent of neurogenesis by means of algorithms acquired before from the mice study. However, we need to bear in mind that it is difficult to generalise from rodents to humans as well as external population validity and the reliability of this study should be increased by replication of randomized controlled trials. Nevertheless, it still provides ‘proof of principle’ data for the future related studies.

There is also emerging evidence that physical activity may have protective effects against the development of neurodegenerative disorders such as AD and dementia, stroke, spinal cord injury and Parkinson’s disease. Scientists now believe that protein synthesised in our brain - BDNF (brain-derived neurotrophic factor) plays a key role in the exercise-induced neurogenesis and synaptogenesis by being a ‘gatekeeper’ for the other effects of exercise. Thereby, by increasing the number of receptor sites linked to the channels (Fig.3), BDNF makes neurotransmitter glutamate, which plays a key role in long term potentiation (LTP – long lasting enhancement in signal transmission between two neurons), learning and memory, more effective. In a study conducted by Carl Cotman from Columbia University elevated levels of BDNF were found in mice that had been running for zero, two, four and seven nights respectively and moreover the relationship between the amount of running done by mice and serum BDNF levels in their brains was established. In addition, the elevated levels of BDNF were not only found in the movement-associated areas (such as cerebellum or basal ganglia) but in the hippocampus as well, which is a key element in the consolidation of memory from STM (short-term memory) to LTM (long-term memory) and spatial navigation. Results from this study inevitably raise the question why are exercise and learning linked? John Ratey in his book ‘Spark’ explains it on the grounds of evolution.
Our ancestors in order to track down, obtain and store the food, needed the ability to learn. Basically it is like a Mobius band – we need fuel to learn and we need learning to find a source of fuel. This straightforward process simply enabled our species to survive and adapt to ineluctable environmental changes. Learning and memory evolved together with our motor functions and logically, lack of movement would mean no real need to learn anything.

A study carried out in Germany in 2007 only enhances the importance of BDNF in cognitive health attainment. Winter et al. assessed learning performance by means of a vocabulary learning task in three conditions: after high impact anaerobic sprints, low impact aerobic running and a period of rest. They found that vocabulary learning was 20% faster and serum BDNF levels were more sustained after intense physical exercise relatively to the other two conditions. Another study examining the effects of short-session intense exercise on cognitive skills used senior participants aged 50-85 years, which time interval is particularly significant in AD susceptibility. Seniors in the first condition were given a memory test after riding a stationary bike and in the other condition after a period of rest. Participants did significantly better in the first condition, recalling more details of the photos they saw before exercising.

However, the majority of people suffering from dementias such as AD, who might need BDNF mostly, are usually incapable of acquiring this neurotrophin by means of exercise due to many age-related obstacles, particularly diseases such as osteoarthritis or varicose veins. Therefore, alternatives for exercise in order to secure high levels of BDNF in the brain have to be put forward. How wide is the spectrum of the other potentially feasible ways? Here I'll present some possible approaches for BDNF augmentation.

DISCUSSION

One family of proteins has received a great deal of attention in AD. BDNF is a member of this small group of neurotrophins which belong to a class of growth factors. This ‘family’ comprises also nerve growth factor (NGF), neurotrophin-3 (NT3), neurotrophin-4/5 (NT4/5) and neurotrophin-6 (NT6). Neurotrophins are proteins responsible for inducing synaptic plasticity, axonal guidance, development and differentiation of the neurons together with their generation (neurogenesis), for preventing apoptosis (programmed cell death) as well as for memory formation and cognition thanks to their signalling capability. Many of them are synthesised in areas of the brain (such as amygdala, entorhinal cortex, hippocampus) that are impacted by AD neuropathology in the early stage of the disease. Moreover, as they are often synthesised away from their site of action, axonal transport is essential for their signalling and it is axonal transport failure, indeed, which is inextricably bound with the neurodegenerative disorders like AD. Therefore, dysregulation of neurotrophins is highly expected in dementias such as AD.

There are two types of nerve growth factor receptors that bind to neurotrophins. Firstly, p75 (low - affinity nerve growth factor receptor – LNGFR), whose role remains controversial – it can activate signalling pathways resulting in the apoptosis. Secondly, Trk (tyrosine kinases) family, consisting of TrkA, TrkB and TrkC. Receptor tyrosine kinases are enzymes capable of
adding a phosphate group to certain tyrosines present on the ‘substrates’ and are located at the cellular membrane. They are activated by the binding of a ligand (e.g. BDNF) to their extracellular domain. TrkB (neurotrophic tyrosine kinase receptor type 2 - NTRK2) is encoded by the NTRK2 gene located on chromosome 9, precisely 9q22, a region that is genetically linked with AD. It binds with high affinity to BDNF, which activates it (as well as to NT3 and NT4/5). There are currently 3 isoforms (different forms) of TrkB found in the mammalian central nervous system (CNS): full-length TK+ and truncated T1 and T2. Expression of the TrkB receptor is regulated by the cAMP/CREB pathway in the neurons.

BDNF is the neurotrophic factor mostly linked with AD. Postmortem studies have confirmed decreased BDNF, pro-BDNF and BDNF mRNA levels in brains of the patients diagnosed with AD (in areas such as parietal cortex and hippocampus) together with mild cognitive impairment (MCI) as well as significant downregulation of TrkB in individual cholinergic neurons (see the AD cholinergic hypothesis described earlier) in the nucleus basalis (NB). Additionally, substantial decrease of full-length TK+ immunoreactivity in the tangle-bearing neurons has been reported.

Amyloid beta ‘Aβ’ is a peptide that is a proteolytic (proteolysis – breakdown of proteins) by-product of the transmembrane protein – amyloid precursor protein (APP), which was found itself to be involved in the neurite growth, survival and post injury repair. However, the accumulation of the neurotoxic APP cleavage product ‘Aβ’ was found to be crucial for AD pathogenesis by causing the synaptic dysfunction and eventually neuroapoptosis. BDNF was found to have protective effects against neuronal toxicity induced by Aβ1-42 and Aβ25-35, completely and partially reversing the toxic action, respectively. Further evidence suggests that TrkB can modulate APP levels and proteolysis. In SK-SY5Y cells (human neuroblastoma cell line) retinoic acid (metabolite of the vitamin A) can increase the expression of full-length TK+ and concomitant treatment with BDNF, can shift APP processing towards α-secretase pathway, which doesn’t produce ‘Aβ’ deposits. Conversely, ‘Aβ’ has been found to reduce BDNF/TrkB levels and to impair TrkB mediated signalling (Fig.4). Furthermore, recent studies have shown that activation of the BDNF/TrkB pathway in mice cells caused dephosphorylation of tau protein, which when hyperphosphorylated, generates intracellular aggregation known as neurofibrillary tangles, the next AD hallmark after ‘Aβ’ composed senile plaques. BDNF/TrkB signalling is of pivotal importance in the modulation of memory processing and storage. TrkB-CREB mutant mice (‘knockout mice’ with inactivated TrkB-CREB genes) when subjected to the Morris water navigation test (hippocampus-dependent learning) and Radial Arm Maze (RAM) test showed severe deficits and partial impairment respectively but no changes in passive avoidance learning, which only enhances the assertion of BDNF/TrkB signalling being involved in complex learning. This behavioural evidence supports the theory that BDNF is essential for the some forms of learning and memory.
The simplest way to promote neurogenesis and avoid neurodegeneration in AD would be just to administer BDNF itself. However, BDNF has a short-life span and its efficacy in the CNS, when taken intraventriculary or intrathecally (introduced under the arachnoid membrane of the brain), is limited as only a minimal amount of BDNF from peripheral administration crosses the blood-brain barrier. Therefore, numerous laboratories are currently trying to find possible ways to deliver BDNF directly into the CNS. Conjugation of the BDNF to polyethylene glycol has been reported to improve its parenchymal penetration in rats. However, there may not be sufficient serum BDNF levels acquired by this method for the larger human nervous system. Moreover, after intrathecal administration, BDNF could actually ‘flood’ the nervous system and lead to weight loss, dysaesthesias (abnormal sensations) and migration/proliferation of Schwann cells (which can form tumours) in the subpial space of the brain. Intraparenchymal infusion of BDNF would be the most direct approach. Nevertheless, it requires further development – an infusion system, capable of preventing reflux, as well as multi-port catheter designs, able to distribute BDNF evenly and widely in the particular brain structure, are indispensable.

Gene therapy is an experimental technique that uses genes to treat or prevent disease. The most common form of gene therapy involves inserting a normal gene to replace an abnormal gene. It is a futuristic and promising approach as it eliminates the use of drugs or surgery. The other forms of gene therapy include inactivating or ‘knocking out’ a mutated gene that is not functioning properly or introducing a new gene into the body. There are two major methods of delivering desirable DNA into the cell: viral and non-viral. The first method uses so called viral vectors such as retrovirus, adenovirus, lentivirus or adeno-associated virus (AAV). It is a plausible strategy as the viruses bind to their hosts and ‘inject’ their genetic material into the host cell as a part of their replication cycle. Therefore, when deprived of their own DNA, they can be used as the ‘vehicles’ that carry the therapeutic DNA. Ongoing clinical trials of in vivo NGF (nerve growth factor) gene delivery in the treatment of AD, using adeno-associated viral
vector serotype 2 (AAV2) to overexpress human NGF (AAV2-NGF) in the nucleus basalis aiming to reduce the loss of cholinergic neurons and stimulate the function of remaining neurons in this area, have shown no adverse effects so far. However, some aspects of this technique, such as accurate targeting and spread of the vector during injection, as well as accurate targeting of the intracranial structures need to be developed through real-time monitoring of vector distribution and real-time imaging of the injection needle position. BDNF gene delivery trials, which are underway, will be based on the new ameliorated method.

As BDNF is normally produced in the entorhinal cortex and hippocampus in adulthood, therapeutic application of BDNF to the entorhinal cortex (Fig. 5) in an animal model was carried out using a viral vector. Transgenic mice expressing a mutant form of APP, which is associated with the early onset of AD, showed significant improvement on two separate hippocampus-dependent tasks. Therapeutic effects of BDNF were also assessed in non-human primates (NHPs) as a prelude to potential future trials on humans (Fig. 6). BDNF gene delivery ameliorated lesion-induced entorhinal cortical neuronal death and improved hippocampus-dependent memory.

However, there are still some risk factors related with the diverse action of BDNF on neuronal functions, which cannot be overlooked and therefore the efficacy and safety of BDNF therapy must be reliably confirmed. Additionally, there are risk factors as well when using the viral vectors, such as their potential toxicity, gene control, immune and inflammatory responses, targeting issues and the possibility of the virus recovering its ability to cause disease, once it is introduced into the patient. The new gene might be inserted in the wrong location in DNA leading to uncontrollable mutations to the DNA and even cancer and such cases have been already reported in the clinical trials. Moreover, transferred genes could be overexpressed, producing so much of BDNF as to be harmful. Furthermore, if the virus regains infectivity, it could be spread from the patient to other individuals in the environment, causing epidemic.

Fig. 5 Potential sites in the human brain for BDNF treatment for specific diseases

Let’s perform a ‘Gedankenexperiment’ (Einstein’s thought experiment). I would like to explore my original and intriguing idea that combines the two ‘holy grails’ of medicine; namely gene therapy and stem cell transplantation. Stem cells have properties to become any one of the more than 200 cell types that make up the human body (pluripotency) and the indefinite capacity to self-renewal while remaining in an undifferentiated state during proliferation. Modified neural stem cells (NSCs) overexpressing human BDNF gene could replace viral vectors and become ‘vehicles’ because of their unusual ability to proliferate. Another possibility would be to transplant NSCs simultaneously with the viral vector overexpressing human BDNF, e.g. AAV2-BDNF. NSCs can differentiate further to form specific cell types, making up the CNS such as oligodendrocytes, in response to many factors, including complex combination of signalling pathways. If we assume that all the differentiation-related criteria are met in the area of the brain with severe AD neuropathology (such as hippocampus or entorhinal cortex), where NSCs are introduced, thanks to the differentiation-inducing BDNF from the viral vector or directly from stem cell ‘vehicles’, beneficial neurons could be generated and the problem would be solved.

This solution, however, would require obtaining NSCs beforehand. They can be obtained from the human embryonic neural stem cells (hENSCs) or neural stem cells derived from the induced pluripotent stem cells (iPSCs). iPSCs are de-differentiated adult somatic cells to embryonic stem cell-like state by the introduction of viral vector(s) that produces several key transcription factors such as Oct3/4, Sox2, Klf4, and c-Myc. Researchers from the Rochester Medical Centre have recently announced the generation of NSCs from iPSCs derived from human skin cells. Of course the treatment involving iPSCs is more appropriate as it doesn’t require the destruction of a human embryo, which raises a lot of ethical issues including so called ‘argument from Playing God’ stating that an act is morally wrong because it is playing God (or ‘playing dictator’ for atheists). On the other hand, all these ethical problems come down to the concept of our humanity, which in turn is distinguished by possessing a more intricate brain and nervous system than the other primates have. Therefore, it can be argued that embryos used for the purpose of the research could not be considered as human beings, as the blastocyst (from which ESCs are extracted) has no central nervous system up to embryonic day 14.
Several studies have reported that BDNF was able to induce NSCs to differentiate into cholinergic neurons (see the AD cholinergic hypothesis described earlier) in vivo and in vitro. Xuan et al. (2008) showed in their experiment that transplanted NSCs could increase the number of cholinergic neurons and ameliorate cognitive skills in rats and BDNF could improve this treatment.

Some drugs have the potential to increase endogenous serum BDNF levels in the brain. Ampakine-induced increments in BDNF levels improved the stabilization of the LTM store in a mouse model with Huntington’s disease. Antidepressants, dietary zinc, lithium and even nicotine were reported to prevent progressive neuropathology and some of them (e.g. lithium) are now undergoing Phase II clinical trials in AD. The main advantage of these pharmacological agents is the fact that either they have been approved by regulatory authorities or are simultaneously undergoing testing for other clinical applications (like ampakines for autism and schizophrenia), thereby making potential clinical trials for other neurodegenerative disorders easier. By contrast, they induce relatively lower amounts of serum BDNF when compared to direct BDNF treatment. In addition, by modulating some cellular signalling pathways, they can generate complex potential effects in the nervous system, which are not fully explored.

CONCLUSION

Alzheimer’s disease is one of the most pressing and yet unsolved problems that future medicine has to face. It is the most common form of dementia. During the course of the disease, synaptic and neurodegeneration take place, progressively impairing a person’s cognitive skills and eventually leading to death. According to the 2009 World Alzheimer’s Report it will affect over 115 million of people in 2050, which is almost 3 times the number of sufferers in 2009.

Neuroprotective effects of BDNF are a recent phenomenon and its exact role played in the central nervous system is still not fully understood. Nevertheless, it has proved in many studies to positively impact areas of the brain affected with neuropathology by inducing neurogenesis, synaptogenesis, differentiation of neurons and preventing apoptosis – programmed cell death. Conversely, decreased levels of serum BDNF were found in people suffering from cognitive impairment with the course of disease. All these features make BDNF an intriguing candidate for a biomarker – a characteristic that can be measured and which reflects the disease process.

By directly influencing downstream cell signalling involved in proper neuron functioning, BDNF could provide beneficial therapy for neurodegenerative disorders arising from different aetiologies. However, problems with administration of BDNF to the central nervous system as well as with effective dosing and safety, have to be overcome. Currently, new techniques of BDNF delivery, targeting the central nervous system and bypassing the blood-brain barrier are well underway.

Gene therapy, stem cells, drugs or even exercise. All of these methods are only a handful of possible ways to increase BDNF levels in the brain affected with neurodegenerative disorders. Exercise seems to be the simplest solution as it doesn’t require any sophisticated
drugs or medical consultation. However, the majority of people suffering from dementias such as Alzheimer’s are incapable of exercising due to many age-related problems. Therefore, alternative therapies using BDNF artificial administration are to be widely taken into consideration, in order to find the proven, safe, universal and most of all efficient future treatment for Alzheimer’s disease. Nevertheless, the nature versus nurture debate is inextricably bound with further development of the techniques such as gene therapy. Nowadays, when scientific understanding of the human genome and the roles of genes are rapidly being elucidated, we have to ask the question whether we should overstep the line marking our humanity in order to create the perfect human race.
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