

Alzheimer's Disease and Neural Transplantation as Prospective Cell Therapy

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Abstract: It has long been recognised that Alzheimer's disease (AD) patients present an irreversible decline of cognitive functions as consequence of cell deterioration in the forebrain cholinergic projection system (FCPS), particularly, in a structure called nucleus basalis of Meynert (nbM). The reduction of the number of cholinergic cells in the FCPS disrupts not just its functions and direct connexions but also the modulation of other systems causing interference in several aspects of behavioural performance including arousal, attention, learning and emotion. It is also common knowledge that AD is an untreatable degenerative disease with very few temporary and palliative drug therapies. Neural stem cell (NSC) grafts present a potential and innovative strategy for the treatment of many disorders of the central nervous system including AD, with the possibility of providing a more permanent remedy than present drug treatments. After grafting, these cells have the capacity to migrate to lesioned regions of the brain and differentiate into the necessary type of cells that are lacking in the diseased brain, supplying it with the cell population needed to promote recovery. The present article aims to review the main aspects of Alzheimer's disease and to explore the use of neural stem cells grafts as alternative treatment for the consequent functional deterioration.

Keywords: Alzheimer's disease, neural transplantation, neural stem cells, animal models.

1 ALZHEIMER'S DISEASE

Alzheimer's disease has been called "the disease of the century" with staggering medical and social dimensions. Epidemiological studies point out that the disease affects 5% of the population over 65. As the life span is being prolonged with the advances in medical science, the incidence of diseases related to aging has dramatically risen. Current demographic projections indicate increased percentages of the elderly in developed countries and similar trends are emerging in the developing nations thanks to the spectrum of interacting social forces and the improvement in quality of life. Age-associated cognitive decline was neglected until recently as a medical entity. However, with the increasing number of aged in our midst, knowledge regarding this common syndrome has been accumulating rapidly and is being translated into strategies for the treatment of the disorder. These strategies of intervention are considerably aided by our freshly acquired understanding of the neurobiology of Alzheimer's disease.

1.1 History of Alzheimer's Disease

Alzheimer's disease (AD) is a progressive neurodegenerative disorder with characteristic clinical and pathological features, although with individual variations for age of onset and pattern of cognitive impairment. Alois Alzheimer first published his observations on the typical

neuropathological changes of the disease nearly a century ago. The work was a case study of a rapidly deteriorating mental illness in a 51-year old woman, Auguste D., who for almost 5 years suffered with increasing memory impairment, disorientation, hallucinations, and personality changes, and died in a completely demented state. A *post-mortem* histological analysis revealed the presence of bundles of fibrils within the neurons and numerous focal lesions in the cerebral cortex.

1.2 Cognitive Dysfunctions in AD

A wide range of cognitive impairments is manifested in sufferers of AD. However, the most common cognitive failure associated with old age and AD is of memory for recent events [1,2]. This is the inability to encode and retain new information, whilst older experiences remain preserved and accessible. This is a prominent feature at the onset of the AD and, arguably, one of the most disabling symptoms causing distress to the patients and those close to them [2].

Progressive impairment of cognitive functions in AD parallels the pathological neural degeneration. In this way, not only memory but also other functions are affected such as attention [3], anxiety and emotional modulation [4]. These conditions give rise to a range of symptoms that cause many problems for the patients and caregivers. Depression, panic, lack of self-care, sleep disturbances, paranoid and delusional symptoms are just a few that can be enumerated.

1.3 Pathophysiology of AD

Modern studies reveal that the findings of Alzheimer were accurate and defined a singular type of dementia. Neuroimaging of patients with AD may show signals of

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atrophy of the brain such as ventricle and sulci enlargement although these features are not always present. Therefore, even with more advanced methods such as functional magnetic resonance imaging (fMRI) [5], these pathological changes were analysed and confirmed from autopsied brain before a definite diagnosis of AD is made.

Pathological hallmarks common to the disease include β -amyloid plaques, dystrophic neurite associated with plaques and neurofibrillary tangles within nerve cell bodies. The exact relationship between these pathological features has been elusive, although it is clear that β -amyloid plaques precede neurofibrillary tangles in neocortical areas [6,7]. Examination of the brains of individuals in the pre-clinical stage of the disease have shown that the earliest form of neuronal pathology associated with β -amyloid plaques resembles the cellular changes that follow structural injury to axons [7]. In some degree, senile plaques and the neurofibrillary tangles are present in brains of normal elderly persons [1]. However, these deposits are considered less toxic than the later-stage plaques.

Braak and Braak, (1991) accessing 83 brains from demented and non-demented patients, postulated and elaborated a scale with stages of intraneuronal lesions, neurofibrillary changes and amyloid deposition [8]. Later on, further studies from the same authors using 2,661 brains of 25-95 year old people (age of death), reinforced their findings and scale of stages [9]. These stages reflected the deterioration that occurs in the AD brain as a gradual progression. Six stages were characterised: mild or severe alteration of trans-entorhinal layer Pre-alpha (trans-entorhinal stages I-II); marked deterioration of layer Pre-alpha in trans-entorhinal and entorhinal cortex and mild involvement of the first Ammon's horn sector (CA1; limbic stages III-IV); destruction of all isocortical associative areas (isocortical stages V-VI) [8].

1.4 Cholinergic System in Alzheimer's disease

In AD several neurotransmitter systems are affected. However, degeneration in the cholinergic system occurs earlier and more consistently than in other systems. The alterations in the cholinergic system that occur in AD patients are thought to be key factors in the cognitive and functional deficits associated with the disease.

The cholinergic hypothesis of geriatric memory dysfunction was generated when several laboratories independently observed that the enzyme responsible for acetylcholine (ACh) synthesis, choline acetyltransferase (ChAT) was depleted in the cerebral cortex of the brains of Alzheimer's patients [10,11]. Since then, several works have repeatedly demonstrated that markers of cholinergic function are decreased in the disease [11-13]. Also, the basal forebrain cholinergic neurons are among the most vulnerable population of neurons in AD [12-15]. The correlation between the progressive and irreversible decline of memory and the loss of cholinergic neurones in the forebrain cholinergic projection system (FCPS) led to the cholinergic hypothesis of cognitive decline in AD.

The diffuse innervation from the FCPS, more specifically from the nucleus basalis of Meynert (nbM) and medial septal

area (MSA), reaches several areas of the brain such as hippocampal formation, frontal and parietal cortex, olfactory bulb and basolateral nucleus of the amygdala [13,16-18]. These structural links suggest a major involvement in the modulation of many aspects of behaviour such as arousal, attention, learning, memory, emotion and information processing [19-22].

1.4.1 Basal Forebrain Projection to the Frontal Cortex

The cholinergic projection neurons of the basal forebrain and upper brain stem, contain six groups of cholinergic projection neurones which were designated Ch1-Ch6 on the basis of cytoarchitectonic criteria and patterns of connectivity [14]. In the basal forebrain are found cholinergic neurones within the medial septal area (Ch1), vertical limb nucleus of the diagonal band of Broca (Ch2), lateral part of horizontal limb nucleus of the diagonal band of Broca (Ch3), and in the nucleus basalis magnocellularis, substantia innominata, nucleus praepopticus magnocellularis (Ch4). The cholinergic neurones of the upper brain stem are found within the nucleus pedunculopontinus (Ch5) and laterodorsal tegmental nucleus (Ch6) [14,23].

Three of these sectors were shown to be involved in learning and memory functions: Ch1, Ch2 and Ch4. Lesions to these areas produced severe mnemonic deficits in rodents [24,25]. The topographical organization of the cholinergic projections to the cortex is limited. Neurones located in the anterior part of the Ch4 region primarily innervate lateral frontal and parietal regions, while neurones found in more posterior areas of Ch4 project to more temporal areas [26].

In general, the anatomical organization of cortical cholinergic afferents suggests a widespread, undifferentiated innervation of cortical areas by basal forebrain cholinergic neurones, rather than activation of a specific cortical region system. Specifically, cortical cholinergic inputs mediate the subjects' abilities to detect and select stimuli and associations for extended processing and to allocate the appropriate processing resources to these functions. Lesions to the primary motor or the prefrontal cortex produced analogous motor and behavioural alterations in rodents and primates. Among the impaired functions after lesions to these areas, primates and rodents present difficulty in shifting responses, poor performance in spatial learning tasks, reduced social interaction, and impaired behavioural habituation [27,28].

1.5 – Animal Models of Neuropsychopathology

Animal models have played a major role in efforts to determine the behavioural and neuronal mechanisms underlying drug effects and have empirically served to develop and test many neuropsychopathological theories. An animal model can be described as an experimental preparation developed in one species for the purpose of studying a phenomena occurring in another. The general argument for the use of animals in behavioural research is that such models allow the testing of specific hypothesis under controlled conditions using methods that are considered either impossible or unethical to use in humans.

In an animal model of a human condition, a material analogy is one of substantive similarity between the animal and human conditions. When trying to understand the

pathophysiology of a disease, it may be desirable to eliminate all but the variable of interest [29]. As models may be used in different ways, it is important to explicit the experimental question and how the model will help to find answers to that question. Animal models of AD have been designed to reproduce various pathological, biochemical and clinical conditions, and to help to elucidate the mechanisms involved in the disease and investigate potential strategies

for treatment (Table 1). Since the neuropathology of AD is both varied and extensive, no truly valid animal paradigm exists. No model can fully mimic the spectrum of behavioural, cognitive, neurochemical and neuropathological changes that are involved in the disease process. Nevertheless, various experimental treatments can simulate some aspects of the disease [29,30].

Table 1. Animal Models of Alzheimer's Disease

	Mechanism	Anatomic target	Negative-Positive	Key refs.
Aging model				
Aged rats	Decline of several neurotransmitter systems	Basal forebrain, hippocampus, etc.	Cognitive decline not consistent; difficult to keep aged animals	[31-33,49]
Neuropathway mechanical destruction				
Fimbria-Fornix transection	Pathway axotomy	Fimbria-Fornix	Unspecific lesions	[36]
Electrolytic	Direct target destruction	NBM, amygdala, medial forebrain bundle	Unspecific lesions	[38,40,42]
Cytotoxin infusion				
Quisqualic acid	Glutamate overexcitation	Basal forebrain	Non-specific lesions	[54,82,221]
Ibotenic acid	Glutamate overexcitation	Basal forebrain	Non-specific lesions	[82,221,222]
Kainic acid	Glutamate overexcitation	Basal forebrain	Non-specific lesions	[221]
NMDA acid	Glutamate overexcitation	Basal forebrain	Non-specific lesions	[82,221]
AMPA	Glutamate overexcitation	Basal forebrain	More specific but not fully selective	[54,82]
Okadaic acid	Phosphatase inhibitor	Ventricles	Mimics biochemistry; memory impairment	[86]
AF64A	Immunotoxin; HACHT mechanism	Several sites	Non-specific lesion effect	[76,77,79]
OX7-Saporin	Immunotoxin; Neuronal antigens + RIP	Basal forebrain	Totally non-selective	[71]
192 IgG-Saporin	Immunotoxin; p75 receptor + RIP	Ventricle + basal forebrain	More cholinergically selective	[70]
Transgenic Genetic models				
Amyloid Precursor Protein (APP)	A deposition, neuritic abnormalities	Hippocampus, cortex, brain stem, thalamus	Partial lesions	[35,90,91]
Tau Protein mutation	Neurofibrillary tangles, filamentous tau aggregate	Amygdala, basal forebrain	Partial lesions	[93]

1.5.1 Animal Models of Senile Dementia and Alzheimer's Disease

Certain neuropathological features can be observed in the brain of aged [31-33] or transgenic animals [34,35]. Other models use mechanical destruction to produce lesions or inactivate cholinergic connections such as the fimbria-fornix axotomy that severs the pathways from the MSA to the hippocampus [36,37].

Another method to induce brain lesions is to infuse agents capable of selective or non-selective destruction of cell populations or even entire structures. Electric current and chronic alcohol ingestion provide alternatives for lesioning procedures [38-42]. However, the lesions produced by these methods are usually non-specific and difficult to use for studies of the pathways involved in neuropathology models.

1.5.2 Aged Animals

One of the most obvious strategies for AD modelling would be, essentially, a geriatric model. Nevertheless, the classical pathological hallmarks of AD, namely plaques and tangles, are exceptionally rare in animals, particularly in small laboratory rodents. Neuritic plaques have been reported in larger animals such as dogs [43], cats [44], bears [45], monkeys [44], and camels [46]. In spite of the rarity of the presence of these pathological markers, aged rats exhibit some of the age-related typical neurochemical changes, such as decline of the levels of dopamine, noradrenaline, serotonin, and cholinergic markers [47,48].

In animal populations, as in humans, age-associated cognitive decline is not a uniform process, and individuals vary enormously in their susceptibility and severity of symptoms. The cognitive impairments observed in aged rats correlate with the degenerative decline of basal forebrain nuclei, and the most severe cholinergic deficits occur in those animals most impaired in tasks of spatial learning, attention and memory [49,50].

The partial validity of an aging model makes it a reasonably attractive proposition. However, this approach is limited as it correlates behavioural symptoms with neurochemical deficits, when correlative studies should not be assumed to imply causation. Nevertheless, manipulation of brain regions and neurochemical pathways enables a more direct demonstration of factors governing behavioural outcomes than seen in aged rats.

1.5.3 Anatomical Targets in Animal Models of Alzheimer's Disease

In rodents, the NBM is the homologous structure to the nucleus basalis of Meynert in humans. This structure is part of the FCPS and has connections to other areas in the brain such as septum, frontal, parietal, and temporal cortex, and hippocampus. Several studies have shown that lesions of the NBM produce reduction of cholinergic parameters such as acetylcholinesterase activity, acetylcholine levels, release, turnover and choline uptake in the frontal and parietal cortices [51,52]. Moreover, excitotoxic lesions of the NBM induce specific memory deficits in rats as evaluated in several tasks [22,26,51,53,54]. NBM lesions may also affect

other aspects of information processing such as attention [55].

Learning and memory disturbances are major characteristics of decreased cholinergic function [22,39,56] and lesions in the FCPS may be involved in other behavioural disturbances in humans and animals such as irritability [57,58], anxiety [20,59], and impaired habituation to novelty [60,61].

Anxiety or reduced exploratory behaviours are usually attributed to mood-related activities controlled by the serotonergic projection system (SPS) [62]. The serotonergic projections from the raphe nuclei are reported to play a modulatory role in the function of the cholinergic basal forebrain projection [63,64]. It seems likely that the degeneration of post-synaptic cholinergic cells in the FCPS may interact and interfere with the activity of the SPS.

Therefore, to reproduce aspects of neurochemical pathology and selectively manipulate the cholinergic system is intrinsically difficult since that FCPS neurons are dispersed amongst a variety of other neuronal systems [18,23,65].

1.5.4 Intraparenchymal Toxin Infusions

The intraparenchymal infusion of toxins is the most used technique to produce lesions of specific structures of brain or CNS. The main objective of this strategy is to destroy or reduce the function of specific cell population that are usually affected in the correlate human pathology. In AD, the reduction of cholinergic function is the main pathological feature and this is the aimed feature in the animal models.

Immunotoxins have been used for years as therapeutics targeting cancer [66,67] or immunosuppression [68,69]. Immunotoxins are conjugates of anti-neuronal monoclonal antibody which selectively target a specific antigen combined with a ribosome-inactivating protein (RIP) [70]. The development of the anti-neuronal immunotoxin 192 IgG-saporin provided a more reliable model of cholinergic degeneration, which can be used to answer fundamental questions about cholinergic biology.

However, the first anti-neuronal immunotoxin compound reported as effective *in vivo* was OX7-saporin, however, it targets any and all rat neurons and was not selective at all [70,71]. After that, 192 IgG-saporin was the first cholinergic-selective anti-neuronal immunotoxin effective *in vivo* and considerably more specific [70]. Based on coupling a potent RIP derived from *Saponaria officinalis* to a monoclonal antibody, 192 IgG, raised against the low affinity nerve growth factor (p75^{NTR}) [72]. The immunotoxin targets the receptor p75 localized on cholinergic nerve terminals in neocortex and hippocampus and on cholinergic cell bodies in the basal forebrain and Purkinje cells in the cerebellum, but not on cholinergic cells found in the upper brainstem [72,73]. Because 192 IgG is specific for rat p75^{NTR}, saporin is supposed not be active in cells not containing this receptor [70]. Whereas 192 IgG-saporin can provide effective focal and clean lesions at relatively short periods (5 months), at longer duration (11 months), necrotic damage and holes in the tissue have been observed suggesting a progressive non-selective damage following the discrete cholinergic loss [53].

The histological effects of 192 IgG-saporin are dependent on purity of the compound, volume of injection, concentration, site of administration and duration post-injection. In addition, incomplete lesions and/or concomitant damage to cerebellar Purkinje neurons hamper the interpretation of the results of 192 IgG-saporin experiments [74].

Knowledge of the biochemistry of acetylcholine (ACh) is an important for producing specific cholinergic depletion. ACh is synthesized from dietary choline and acetyl-coenzyme A (Acetyl-CoA) derived from glucose metabolism in the mitochondria. The enzyme catalysing this reaction is choline acetyltransferase (ChAT) and the synthesis of ACh is controlled by the high affinity choline transport system (HACHT) localized in the cholinergic terminals [75].

Ethylcholine aziridinium (AF64A) was once hailed as a selective cholinotoxic agent [76]. AF64A combines a choline structure that is recognized by the HACHT system and the cytotoxic moiety of aziridinium [75]. Due to the choline similarity of AF64A, HACHT accumulates it, and once inside the terminal the highly reactive aziridinium induces a pathological cascade that results in cell death [76]. In other words, AF64A is a "Trojan Horse" that masquerades as choline and releases a killer when inside the terminal.

AF64A specificity is dependent on a multitude of interdependent variables such as purity of the compound, dose, concentration, injection volume, and site of administration [76]. The anatomical action of the substance is governed by site of injection. Functionally, it induces deficits in the performance on several memory related tasks [75,77,78]. Although, AF64A selectively lesions cholinergic input to the hippocampus, it produces an incomplete lesion (less than 50%) [77] and cannot be injected directly into tissues due to local non-specific effects [79].

1.5.5 Excitotoxin Infusions

The use of excitotoxins to produce cholinergic depletion is by far the most common method in neuroscience. Stereotaxic injections of excitotoxins, such as *N*-methyl-D-aspartate (NMDA), kainic, ibotenic, quisqualic, okadaic, folic and α -amino-3-hydroxy-4-isoxazole propionic acid (AMPA) have been widely used to produce intracerebral cholinergic damage. These compounds act on glutamatergic receptors, destroying neuronal cell bodies, by a process long known as excitotoxicity where these glutamatergic agonists produce a massive and sustained depolarisation in postsynaptic cells to the point that the cell membranes and their associated ion pumps collapse and lose their ability to maintain cell homeostasis. Thus the cells die by excessive stimulation [80].

Several studies have reported comparisons of the effects of these compounds either behaviourally, biochemically or histologically [53,54,81-83]. The variability of excitotoxic potency and specificity of these compounds may be related to different glutamatergic receptor subtypes. Nevertheless, AMPA has been reported to be consistently more selective in cholinergic depletion than all other excitotoxins in use, when injected into the NBM or MSA [54,84,85]. However, it is considered less selective when injected into other areas or in larger amounts [82].

Recently, chronic infusions of okadaic acid into the ventricles have been reported to produce severe memory impairment, beta-amyloid plaque-like formation and elevated Tau protein phosphorylation which are arguably major hallmarks for AD characterization [86].

In AD, the wide range of cognitive impairments is related to the degeneration of cholinergic neurons in the NBM and MSA making these the sites of choice for lesion models of cholinergic pathologies. However, the presence of other neuronal systems running almost parallel to the FCPS makes it a difficult target. There is a GABAergic projection system with cell bodies originating in the MSA connecting to interneurons in the hippocampus [87]. Also, the serotonergic projection system (SPS) from the raphe nuclei is reported to play a modulatory role in the function of the cholinergic basal forebrain [63,64,88]. The extent to which excitotoxic forebrain lesions can induce damage or influence other systems has yet to be resolved, but it is likely that interactive effects have been underestimated. Moreover, in AD patients it is not only cholinergic cells that are deteriorated in those regions.

1.5.6 Transgenic Models of AD

Genetic approaches may be the most recent and powerful instrument to elucidate psychiatric, neurological disorders, and human and animal behaviour. In recent years, brain researchers have been provided with several transgenic animal models of diseases. The focus of these strategies is the possibility to design and engineer genetic changes that are transmitted to subsequent generations providing permanent experimental models. The traditional models of AD have concentrated in the neuropathological features such as neuronal damage and cholinergic depletion. Although informative, these models were considered too restricted, or acute (rather than gradually degenerative), or failing to produce AD-like behavioural deficits. Therefore, not surprisingly, the transgenic animal models of AD were eagerly anticipated and have been extremely informative.

Although there are features in AD that are recognised to be as important about the process of neurodegeneration, the most significant feature of transgenic animal models of AD is the over expressed amyloid phenotype [89]. Several mice strains have been shown to manifest pathological amyloidosis and significant behavioural deficits on memory and learning [90,91], neophobia [92], and accelerated taste aversion extinction [93].

Whereas the transgenic approach has proven to be useful and informative on aspects of cell neuropathology that underlies human AD, it has yet not produced convincing replication of processes that could create a perfect animal model for AD.

2 PHARMACOTHERAPY IN AD

To date, treatment for AD has been exclusively pharmacological. Once is thought that a deficiency in the cholinergic neurotransmission leads to the functional deficits and clinical manifestations observed in AD patients, this neurotransmitter system has been the prime target of pharmacological treatment. Therefore, the presence of acetylcholinesterase (AChE), the enzyme responsible for the degradation of acetylcholine, is decreased in brains of

persons with AD [10]. Another enzyme that degrades acetylcholine is butyrylcholinesterase (BuChE). Whereas BuChE is found only at low levels in the normal brain, its levels are significantly increased and it is more widely distributed in the brains of persons with AD [94]. Consequently, blocking the activity of BuChE and AchE could, theoretically, lead to the slowing of the progression of the disease, although the relief effect is temporally.

Several compounds have been used for pharmacological treatment of AD. However, the agents that exploit the mechanism of AchE and BuChE are currently the only class of medication approved with this objective. Generically called cholinesterase inhibitors (ChEI), tacrine, donepezil, rivastigmine, and galantamine are drugs that act in the AchE and BuChE mechanism. All of them produce behavioural and cognitive effects on AD patients [95-97]. These drugs bring also risks of side effects. For instance, tacrine is associated with high hepatotoxicity [96], donepezil is selective for AchE but has peripheral side effects, rivastigmine is unspecific for AchE or BuChE and causes nausea, vomiting, intestinal problems, abdominal pain and dyspepsia [98], and galantamine has similar side effects to rivastigmine [99].

Other pharmacological approaches have long been investigated for relief of the symptoms of AD and slowing the disease progression, such as antioxidants, monoamine oxidase inhibitors, anti-inflammatory agents, oestrogen and ginkgo biloba. These agents may have some limited utility as potential adjuvant therapies to the ChEI treatment. However, to date, there is no convincing evidence of efficacy for any of these agents.

There are also drugs that can interact indirectly with the cholinergic system and provide significant effects. Nootropic drugs are a class of psychotropic agents developed in the search for a gamma amino butyric acid (GABA) analogue. Piracetam was the initial compound developed of this class, but failed to demonstrate any GABA-like activity. However, it had a therapeutic effect centrally on nystagmus in rats [100] and the capacity to improve learning in the Y-maze and water maze in normal, aged and alcoholic rats [101,102]. Piracetam showed some positive effects in clinical trials in cerebral arteriosclerosis patients and stimulated other studies for senile dementias and AD [103]. Further studies of nootropic drugs are still needed, but it appears that this class of drugs may exert some positive effects on AD, although it is of little clinical value in psychiatric illnesses such as schizophrenia and depression.

A number of other agents have been investigated and are showing promise in early or late trials. Intense areas of research are focusing on agents that prevent beta amyloid build-up, its toxic effects on nerve cells, or other mechanisms of the disease process including action on other neurotransmitter systems. Cerebrolysin is an example of drugs based on nerve growth factor (NGF) therapy and has shown to improve mental function in clinical trials [104]. Insulin and insulin growth factors have been studied as a method to prevent β -amyloid accumulation [105-107]. Treatment with antioxidants is another promising approach for slowing disease progression and exploits the rationale that oxidative damage may be responsible for the cognitive

and functional decline observed in AD. Indole-3-propionic acid is a natural antioxidant agent (melatonin) that interferes on enzymes which may change the biochemical processes involved in AD [108,109].

Studies investigating the use of antibiotic drugs such as β -cycloserine [110] have demonstrated some low impact effect on recovery from the disease. N-methyl-D-aspartate (NMDA) blockers, such as memantine, have also been reported to reduce severe dementia and improve memory [111]. Its action, however, still demonstrates limited action in recovery of memory function. Yohimbine is a potent selective α_2 -adrenoceptor antagonist with predominant pharmacological use in the treatment of male erectile dysfunction. However, it has been tested in AD patients producing increased agitation, anxiety and excitability [112] but improved social interaction. Clinical correlates of reduced serotonin (5-HT) in AD remain unknown. It has been suggested that there is a complex link between aggression in AD and central serotonergic dysfunction which interacts with cognitive impairment [113]. Antidepressants known as selective serotonin reuptake inhibitors (SSRIs) may be particularly effective in relieving depression, irritability, and restlessness associated with AD. Preliminary studies with SSRI agents such as citalopram, fluoxetine or paroxetine, showed reduction of irritability [114] and no side effects on cognitive impairment [115].

All these compounds offer different approaches to the treatment of AD. However, none of them is able to offer much symptom relief or permanent efficacy. Also, the cost for treatment with these drugs is high and side effects are prevalent. The ChEIs have been shown to delay the progression of the symptoms, thus extending the patient's time in a less impaired state, which is a positive effect of treatment, but not a long lasting gain.

3 NEURAL TRANSPLANTATION

Neural transplantation is a promising strategy for treatment of several CNS pathologies that offers the prospect of permanent cure. It also brings the possibility for other techniques such as cell carriers for gene therapy [116,117] or NGF delivery [118]. Prior to transplantation, cells can be manipulated *in vitro* and transfected with genes coding for functionally relevant proteins to assist the repair of the diseased brain or used as mini-pumps synthesizing and delivering compounds to the deficient brain. The most obvious possibility is to use neural transplantation as a technique for cell replacement therapy whereby the cells would occupy the place or the function of dead or degenerated cells.

The potential goals for therapeutic use of cell transplantation in neurological disorders are ambitious. Several animal models of different clinical human conditions are already being studied as target pathologies for neural transplantation such as Alzheimer's [119], Parkinson's [120], and Huntington's diseases [121,122], ischemia [123,124], stroke [125,126], brain tumour [127], spinal cord injury [128,129], brain lesion by drugs or alcohol ingestion [39,130], brain concussion [131], epilepsy [132,133], brain decline by aging [134] or consequences of high radiation levels [135,136].

3.1 History of Neural Transplantation

Over a hundred years ago, the first attempts at grafting CNS tissue in adult brain were published. In the pioneering works of Walter G. Thompson in 1890 [137], cats and dogs were used to investigate the survival of grafted tissue. Thompson wrote:

“Of course, I had no expectation of being able to restore abolished function by the operation, but the question of vitality of the brain tissue and the course of its degeneration is a subject which is of very wide interest.” (Page 701, paragraph 1).

However, it was only in the last 30 years that the technology required for successful transplantation of CNS tissue was being developed. In 1976, a work published by Lund and Hauschka showed that superior collicular fragments transplanted from foetal to newborn rat brains developed complex internal organization and received visual afferents from the host brain [138]. This work highlighted the possibility of intracerebral CNS-derived transplants may be able to integrate with damaged neural tissue.

Other works from the same decade showed successful transplantation of immature central and peripheral neurons into the CNS [139,140]. At that early stage, success in neural transplantation was a limited matter of transplant survival. However, it was becoming clear that implants of neural tissue into the damaged CNS could ameliorate symptoms of motor and cognitive dysfunction. Then, just as now, the mechanism of recovery remained elusive.

When the donor tissue is immature, from foetal or neonatal sources, and the site of implantation is richly vascularised, the chances of transplant survival are hugely increased [80]. Therefore, early grafting studies transplanted solid tissue into either the third or the lateral ventricles where it could be bathed by the cerebrospinal fluid [141] or in a wound cavity created in the entorhinal/occipital cortex of developing rats where the presence of neurotrophic factors could influence graft survival [142]. However, transplantation of solid tissue into deeper areas, such as the basal forebrain, would undoubtedly risk damaging host tissue, increase the possibilities of immune response, and cause graft rejection.

In the early 1980's, Björklund and co-authors developed the method of cell suspension for implanting into deep brain sites [143]. The standard cell suspension procedures (dissection of tissue in sterile conditions; digesting of the tissue by enzymes such as trypsin to break cell adhesion; washing and inactivation of the enzyme by adding a trypsin inhibitor; DNase-washing to avoid cell clumping; mechanical dissociation and cell viability counting by Trypan blue exclusion in a haematocytometer) and stereotaxic placement techniques were successfully used. In this method the graft is in direct contact with the host neuropil and does not require vascular support or access to CSF spaces. Despite the progress that this method brought to the field, several problems had to be solved. Dissociation methods may destroy some of the inherent structures of the donor tissue making it unsuitable for more sensitive cells [80]. In this case, the amount of tissue needed to prepare a

reasonable amount of cells in the cell suspension was much greater.

A number of factors including age of donor, and recipient, and site of implantation may influence the success of graft survival. Another factor is the presence of major histocompatibility complex (MHC) class I and II cell surface antigens. Multicellular organisms have the ability to recognise self from non-self, and can reject non-self tissue. Class 1 and 2 cell surface antigens are involved in recognition of non-self tissue and activation of T helper cells. When the transplant is lacking these classes of cell surface antigens, the risk of immune response and rejection is greatly reduced. The blood brain barrier and lack of lymphatic drainage in the CNS prevents the passage of plasma proteins between the CNS and the lymph system, [144] and it is the reason why the brain was called an “immunologically privileged” site. Nevertheless, studies of neural transplantation have indicated that this is a controversial issue [145] and immunosuppressant therapy protocols were adopted aiming to help the studies of cell survival especially with cross-species grafts [146].

Cyclosporin A (CsA) has been shown to be effective for protecting neural xenografts [147-150]. CsA acts by binding to the intracellular immunophilin receptor cyclophilin, inhibiting the enzymatic activity of calmodulin-dependent phosphatase calcineurin, which is involved in several forms of cell death [151]. It has been also reported to have protective properties, increasing the survival of dopaminergic cells during the preparation of nigral suspensions for grafting [147].

3.2 Initial Clinical Trials in Neural Transplantation

To date, most clinical studies in neural transplantation have focused on the idiopathic pathology of Parkinson's disease (PD) and the prospectives are promising [152,153]. PD is a neurodegenerative condition of unknown aetiology that produces crippling motor symptoms, poverty, and slowness of voluntary movement, tremor, and rigidity. All these symptoms are progressively debilitating and associated with a relatively selective degeneration of the dopaminergic nigrostriatal pathway [154,155]. Some drugs can effectively diminish these symptoms but only in early stages. The most commonly used drugs in PD are those that support the remaining dopaminergic neurons in the pathway and dopaminergic agonists, which directly activate dopamine receptors [156]. However, the efficacy of these drugs declines as the disease progresses insidiously. The dosage of these drugs is then increased and, in consequence, side effects such as dyskinesias, unwanted movements, and “on/off” motor fluctuations become more frequent. As a result, patients taking these drugs after five to ten years have major problems, some even worse than the disease itself [156].

The possibility of developing a more permanent treatment for brain pathologies like PD pointed research towards more direct interventions. The clinical trials carried out by Backlund's group and published in 1985 was the first clinical trial on neural transplantation [157]. They implanted chromaffin cells from adrenal medulla (AM) which are extremely rich in catecholamines being possible to be forced

to produce higher levels of dopamine [158]. Chromaffin cells share the same embryologic origin from the neural crest with SNC cells and can extend neuronal-like processes *in vitro* when NGF treated [159]. After grafted into the ventricular space, dopamine secretion is augmented, suggesting that transplantation causes a shift in the relative amounts of catecholamines produced [160].

The results in animal models of the disease showed positive changes [161] with reduction of the symptoms produced by lesion. However, for Parkinsonian patients the benefits were less than modest. In the Goetz *et al.* [162] two-year follow up multicenter study, the evaluation of 56 patients treated, 18% were dead after 2 years, 22% of the remaining patients, developed persistent psychiatric morbidity and 19% improved on global rating scores. Other studies have demonstrated more alarming negative results with 100% showing non-improvement [163,164]. A recent review reported at least 17 deaths of various cause, out of 231 operations, within the first 5 years of the operation [165]. These disappointing results and the balance of morbidity and mortality as well as modest and transient improvements, have generated a consensus that the AM graft was no longer justified [80].

3.3 Neural Transplantation of Embryonic Tissue

The unsatisfactory results obtained from AM transplants led to the development of other techniques and surgical procedures and the next step in neural transplantation research was the use of embryonic tissue transplants. In contrast to AM grafts, the first clinical trial only took place a decade after the initial basic experiments in animals had started [80]. Apart from the technical and ethical issues that this procedure raised, the results in animal models were far better than with AM grafts.

Transplants of embryonic tissue using animal models indicated the possibility to alleviate Parkinson-like symptoms resulting from dopaminergic damage or cell loss [166-168]. The initial clinical trials showed only modest relief after transplantation and no clear demonstration of graft survival [169]. Subsequently, reports of graft survival and dopaminergic reinnervation of the striatum were definitely demonstrated by histopathological studies [170].

Recent clinical studies have been carried out and long-term follow-ups show that in many cases the implantation of neuronal cells is effective. The first controlled clinical trials of foetal tissue have shown encouraging results. In a blind study with 40 patients assigned to two groups, transplanted and sham grafted, it was shown that human embryonic dopamine-neurons transplants survive in patients with severe PD and resulted in some clinical benefit in younger but not in older patients. However, it was reported that 15% of 33 patients who ultimately received transplants and survived for as long as three years after surgery developed dyskinesias and dystonia [152]. At the moment, over 200 PD patients have been grafted with human embryonic mesencephalic tissue [171]. Overall, although embryonic tissue transplants provided good recovery in many measures, all of the patients remain Parkinsonian [172].

3.4 – Neural Transplantation in Alzheimer’s Disease

Although there are no reports at the moment of clinical use of embryonic transplants in AD patients, several works have demonstrated that cholinergic-rich cells of embryonic origin can improve the performance of animals with cholinergic depletion [173-178].

Embryonic tissue collected from several different parts of the brain such as hippocampus [179], septal area [180,181], locus ceruleus [182], ventral and basal forebrain [19,183], ventral mesencephalon [167,168] have been demonstrated to promote regeneration and connectivity. Tissue from cholinergically rich areas grafted into the brain of lesioned or non-lesioned, newborn [179], adult [51,179] or aged animals [180] showed axonal growth.

In rats with damage to the FCPS, transplantation of embryonic ventral forebrain to neocortex improved the performance in passive avoidance and elevated plus maze [184], the radial arm maze [39,174], multi-choice reaction time task [176] and in the Morris water maze [175].

3.5 Neural Stem Cells

Most of what is known about the adult brain suggests that it is a stable structure meaning that neurons can only be generated at discrete times during development. However, several groups have challenged this view showing that neurons are born in the adult mammalian brain [185,186]. The developing brain represents a spectrum of differentiation, encompassing at one end mature differentiated cells with no ability to divide and at the other end a rare, self-sustaining population of stem cells that have the capacity to give rise to all cells of the CNS.

However, the fact that there is neurogenesis in the adult mammalian brain does not guard it against degeneration by age, injury, or disease. And although there is a reduced production of new cells, the brain cannot rebuild itself alone. Therefore, implantation of stem cells might help in this function. Stem cells are self-renewing multipotential cells with the developmental capacity to give rise to all cells of a particular tissue as opposed to progenitor cells that define a cell committed to a determined and restricted fate [187,188].

Neural stem cells (NSC) implanted in the CNS have the potential to develop into neurons, astrocytes, and oligodendrocytes, as well as to self-renew [187]. This capacity to differentiate into multiple cell types defines the concept of multipotentiality [187,189]. Moreover, NSCs have other important characteristics. When implanted into the brain, NSC tend to follow host signals and migrate [190,191]. Multipotentiality and migration mechanisms are regulated predominantly by environmental signals [190]. Several observations suggest that the mature CNS retains at least some of the developmental guidance cues [192-194].

One of the aims of stem cell research is to generate cell lines that can be maintained and expanded indefinitely *in vitro* and be readily available for transplantation. Also, these cell lines must be capable of repairing the damage present in the host brain. The most promising scenario so far is the use of immortalised CNS-derived neural stem/progenitor cells lines.

3.6 Immortalised Neural Stem Cells

The most appealing characteristic of the stem cells is the potentiality to differentiate into multiple lineages of cells. This capacity is associated with the possibility of maintaining cells in culture ready to be transplanted, rather than harvesting them afresh from foetuses each time they are needed. The use of cell lines grown in the laboratory offers the most spectacular prospect of future treatment for common diseases.

Heterogeneous populations of primary cells can be dissected from foetal brains, and later on expanded *in vitro* for transplantation. However, primary cells undergo a finite series of divisions prior to differentiation and/or senescence, thereby limiting the number of cells available for grafting. The derivation of immortal cell lines offers an attractive alternative to the use of primary tissue. The use of embryonic tissue to obtain primary cells for transplantation has also ethical problems associated with the use of human embryonic tissue and practical problems linked to the fact that it is a heterogeneous population of mixed cells.

Cell lines can occur naturally like the PC12 cell line derived from a rat pheochromocytoma [195] or be produced *in vitro* by the introduction of so-called 'immortalising genes' [196-199]. The rationale for immortalisation is to induce the cells to stop the progress of natural developmental programmes that cause cell death, keeping the cells in a continuous cycle by the introduction of a gene that will reprogram the cell cycle sequence [198,199].

However, the cell lines that go into continuous cycle have the tendency to keep growing and dividing. For transplantation, it is necessary that the cell line stop dividing at determined conditions. This has been engineered by the introduction of genes that allow the cells to divide under certain conditions, but not others, a procedure termed "conditional immortalisation" [189,200-202]. The introduction of the temperature sensitive (ts) SV40 large-T into the cellular genome allowing the cells to divide and proliferate at the permissive temperature of 30-33°C. However, when the temperature is raised to the non-permissive level of 37-39°C, the gene down regulates and cells cease to divide and can be induced to differentiate [200].

Sinden and colleagues [189] developed a conditionally immortalised stem cell line, the Maudsley hippocampal clone 36 (MHP36), using the temperature-sensitive oncogene SV40 large-Tag. The cell line was derived from the H-2K^b-tsA58 'immortomouse', a mouse genetically engineered to constitutively express the temperature-sensitive mutant simian virus 40 (SV40) large-T-antigen (Tag) under control of interferon-inducible H-2K^b promoter as part of its genome [200]. This cell line has been shown to be functionally effective in many animal models of brain damage such as 4 vessel occlusion ischemic insult [189], reverting spatial learning deficits to those of control levels. In addition, MHP36 cells migrate and selectively repopulated the lesioned hippocampal CA1 pyramidal layer [189].

Other important properties have been demonstrated by this cell line showing advantages over primary, genetically modified or trophic graft approaches. These cells are

conditionally immortal expandable at low temperatures (30-33°C) *in vitro*, ceasing to divide after grafting and differentiating into mature cells [189]; migratory after grafting, moving specifically to regions of brain damage [19,189,203]; multipotent, differentiating into neurons, glia or oligodendrocytes *in vivo* [19,189] and *in vitro* [19,189,204]; multifunctional, reducing deficits in different models of brain damage such as excitotoxic lesions in marmosets [124], stroke [125,126], age-associated memory impairment [123], cholinergic excitotoxic lesions in rats [178], and radiation-induced myelopathy [135];

3.7 Human Stem Cell Lines

The use of human cells for research or therapeutic purposes has been restricted in part by their limited proliferative potential. Normal human cells have a limited life span and undergo cellular senescence after a finite number cell division before permanently exiting the cell cycle and becoming senescent [205-207]. For many years, the standard method for obtaining immortal human cell lines was to derive them directly from tumours. However, the progress of the research of stem cells is changing this scenario very quickly.

Human NSC are primordial, uncommitted cells postulated to give rise to more specialised cells and defined as mitotically competent, multipotent and specially self-renewing [185,187,208-210]. The isolation and expansion of human NSC has important potential for clinical applications and is considered to be the "Holy Grail" of the neural transplantation field. Recently, several laboratories have reported to have isolated and expanded long term cultures of human neural stem and precursor cell lines [196,210-214]. However, few of these groups have demonstrated behavioural assessment of transplanted animals.

3.8 Mechanisms of Action of Neural Transplants

It is clear that grafts can produce functional recovery in animal models of brain damage. There are also some reports of recovery for patients implanted with embryonic tissue. However, the mechanisms of recovery are not completely understood. It is very likely that more than one mechanism is involved in the processes of recovery.

The main objective of neural transplantation is to achieve cognitive and functional recovery of the damaged CNS. There are several possible mechanisms by which these aims can be fulfilled. The fundamental rationale of neural transplantation was to supply the damaged brain with substitute cells to replace the lesioned, dead or missing cells. The mechanism involved in the processes of functional changes in this rationale is called reconstruction and occurs when the transplanted cells or tissue form functional connections with the host cells from the lesioned area. This is one of the mechanisms used to explain functional and neurophysiological recovery after neural transplantation, but it is not the only one. A variety of neurotrophic factors and cell signals may be involved in the process of sprouting and re-innervation of grafted cells and host tissue and some of these mechanisms need to be emphasized.

Table 2. Sources of Tissue for Cell Transplantation

Primary foetal cells (rodent E12-18; human foetal 8-14 weeks; porcine xenografts)	
Advantages	Disadvantages
Good survival in animal models	Ethical issues with human foetal tissue
Variable survival in patients	Practical problems of supply with human tissue
Functional efficacy in animal models	Mixed populations of cells at different stages
Variable efficacy in patients	Poor integration into host brain
	Possible overgrown
	Possible rejection of allo- or xenografts
	Limited chances to screen clinical cells for viruses
Stem cells (Human ES cells up 2 weeks; rodent ES cells within 1 week; Human Foetal 8-12 weeks; rodent E11-12; adult bone marrow, post-mortem CNS, umbilical cord, blood)	
Advantages	Disadvantages
Cells screened thoroughly for viruses	Control over cell division is essential, to reduce high risk of tumourigenesis
Once obtained, ethical problems reduced	Control over oncogene activity may be required to prevent abnormal cell division, and expression of abnormal/unstable karyotypes
Cell differentiation guided by the host brain	Genetic abnormalities from donor cells may be amplified
Unlimited supply via conditional immortalisation	Variable survival and efficacy in animal models
Cells grown in culture and banked maintaining purity and uniformity	Unknown requirements for immunosuppression
Migration and flexible integration into the host brain	
Potential for autologous grafts	

One of these mechanisms involves effective release of substances from grafts. Implanted cells or tissue must have capability to carry and/or synthesize and release substances into the host tissue in the vicinity of the graft. These cells may be engineered to deliver substances capable of reducing cell degeneration and inducing neural regeneration of the damaged CNS [215]. For example, cells genetically modified to secrete NGF implanted into rats' brains prevented the degeneration of axotomised cholinergic neurons. In the septum, immunoreactivity to the low affinity NGF receptor p75 and to ChAT was enhanced; and hippocampal AchE levels were increased, indicating restoration of cholinergic function [216].

The indirect stimulation of neurotrophic release from the host as a result of implantation of stem cells is another mechanism involved in the structural reorganization of the damaged brain. This plasticity mechanism of NSC grafts was observed in stroke lesioned animals that presented high

apolipoprotein E (apoE) expression [217]. ApoE is a lipid transporter protein that has been associated with plasticity, regeneration and brain development. Therefore, NSC grafting promoted plastic changes by the host neurotrophic release. Also, in a lesion model of AD, grafted animals presented a down regulated expression of Oct6 (Oliveira et al, unpublished observations). Oct6 is a developmental transcription factor involved in regulation of the myelin gene expression [218]. This protein is over expressed in lesioned animals while it is down regulated in NSC grafted animals.

Grafted cells may also provide a substrate for axonal growth to new or old destroyed targets. This mechanism is known as bridge and involves implantation of cells able to promote rescuing and guidance of damaged cells. Glial cell lines rich in developmental factors may stimulate the formation of host glial cells [219] or cells implanted may simply work as scaffolding structures for axonal growing [220].

Research into mechanisms of graft action is of crucial importance because it may bring insights on the search for better use for cell transplantation therapies. The effects observed in neural stem cell grafts may be result of one or a sum of several mechanisms. Therefore, it is very important to consider all factors involved from behavioural and neurological effects to cellular and physiological changes.

Table 3. Mechanisms of Graft Action

Mechanism	Description
Replacement	Grafted cells extend process into the host brain and form synaptic connections replacing lost cells
Scaffolding	Grafts supply the host with a substrate on which axons may grow and re-innervate distant targets
Neurotrophic	Cells release trophic factors and/or stimulate neurotrophic release from the host, or results from a host inflammatory response
Neuroendocrine	Grafts release neurohormones into local blood vessel, which are transported to the CNS through the host circulatory system
Paracrine	Release neurotransmitter in a non-specific manner producing effects on host cells
Non-specific	Wield actions as a result of direct or indirect damage at the site of implantation, independent of type and state of graft at the target site

CONCLUSIONS

The causes for brain damage in AD are varied and not completely known affecting different areas of the brain and cell types in particular ways. Thus, the discovery of the causal factors is of extreme importance and would lead to a major impact in clinical practice. With an estimate of over 37 million people worldwide affected by this disorder (The World Health Report, WHO, 2001) the total costs just in the United States were estimated to be over US\$ 1.75 billion for the year 2000 with prospects of great increase in the next 20 years. Consequently, a possible cure would be likely to be worth more than five billion US dollars.

The symptoms present in AD have been treated almost exclusively by pharmacological approaches which are minimally effective, temporary, and can cause secondary undesirable effects. The use of neural transplantation for the treatment of this disorder is still in a pre-clinical level. However, it raises promising prospectives for permanent treatment for this and other neurodegenerative diseases. Neuronal replacement by intracerebral transplantation of primary foetal grafts is being currently clinically explored as strategy to promote functional recovery from brain diseases such as Huntington's and Parkinson's disease.

Although some progress has been achieved, there are still several problems related to the use of foetal tissue grafts. From a practical prospective, foetal material donated from routine suction abortions are typically fragmented, and potentially more imprecise than those achieved in controlled

laboratory conditions. Suspended cells from embryonic material are derived from heterogeneous cell population and, in consequence, two grafts will never be the same, being inherently of poor reproducibility compromising the understanding of the mechanisms of functionality. Donated tissue needs to be assessed for infections, which is a time consuming and complex process for the time constraints of neural transplantation. Time between donation and transplant is also a very important matter. Poor survival of neuronal cells has required the use of multiple foetal donors for each recipient in all successful clinical trials to date. The simultaneous collection of multiple donors all within the correct developmental age window makes the practical strategy of developing a feasible clinical transplantation programme very difficult.

Therefore, from the clinical point of view, an ideal situation would be if cells could be available and supplied at the time needed. In spite of the fact that the use of stem cells carries similar ethical implications as embryonic tissue, it has the possibility to be maintained *in vitro* indefinitely, so that they may be always available for implantation. This is the major objective in the stem cell research field. The multipotentiality of neural stem cells raises the possibility that a relatively smaller number of cell lines may be capable of repairing different kinds of brain damage. The repair ability of stem cells and the possibility of permanent treatment is, so far, the most interesting approach in the search for treatments to alleviate brain damage in AD. Stem cell grafting for the treatment of AD is still at a preclinical level. However, it raises promising prospects for permanent treatment for this and other neurodegenerative diseases.

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